JUL 25 1924

THE JOURNAL OF GENERAL PHYSIOLOGY

Medical Lib.

EDITORS

JACQUES LOEB W. J. V. OSTERHOUT

VOLUME VI, No. 6 JULY 20, 1924

PUBLISHED BIMONTHLY

AT MOUNT ROYAL AND GUILFORD AVENUES, BALTIMORE, MD.

BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

COPYRIGHT, 1924, BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

Entered as second-class matter November 25, 1918, at the Post Office at Baltimore, Md., under the Act of March 3, 1879.

Made in the United States of America

PUBLICATIONS OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

THE JOURNAL OF EXPERIMENTAL MEDICINE

EDITED BY

SIMON FLEXNER, M.D. PEYTON ROUS, M.D.

The Journal of Experimental Medicine is designed to cover the field of experimental medicine. It is issued monthly, two volumes of over 600 pages each appearing in a year. The subscription price per year (two volumes) is $5.00, payable in advance. Single copies cost 75 cents. Remittances should be made by draft or check on New York, or by postal money order, payable to *The Journal of Experimental Medicine*, Mount Royal and Guilford Avenues, Baltimore, Md., or Avenue A and 66th Street, New York, N. Y.

THE JOURNAL OF BIOLOGICAL CHEMISTRY

EDITED BY

STANLEY R. BENEDICT, New York, N. Y. LAFAYETTE B. MENDEL, New Haven, Conn.
H. D. DAKIN, Scarborough, N. Y. DONALD D. VAN SLYKE, New York, N. Y.

The Journal of Biological Chemistry is designed for the prompt publication of original investigations of a chemical nature in the biological sciences. It is issued monthly. Each volume consists of as many numbers as are required to make a total of approximately 800 pages. The subscription price is $5.00 per volume to subscribers in the United States and Canada, payable in advance; $5.25 to those in other countries. The number of volumes issued yearly averages four. The price of single copies varies; it is given on the back cover of each number. Remittances should be made by draft or check on New York, or by postal money order, payable to *The Journal of Biological Chemistry*, Mount Royal and Guilford Avenues, Baltimore, Md., or Avenue A and 66th Street, New York, N. Y.

THE JOURNAL OF GENERAL PHYSIOLOGY

EDITED BY

JACQUES LOEB, New York, N. Y. W. J. V. OSTERHOUT, Cambridge, Mass.

The Journal of General Physiology is devoted to the explanation of life phenomena on the basis of the physical and chemical constitution of living matter. Information regarding contributions and subscriptions is given in full on the back cover.

STUDIES FROM THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

The published results of investigations conducted in the Institute, or under its grants, are republished at irregular intervals in volumes designated *Studies from The Rockefeller Institute for Medical Research*. The subscription price is $2.00 per volume, payable in advance. A special rate of $1.00 per volume is made to those who subscribe to *The Journal of Experimental Medicine*, *The Journal of Biological Chemistry*, or *The Journal of General Physiology*. Remittances should be made by draft or check on New York, or by postal money order, payable to The Rockefeller Institute for Medical Research, Avenue A and 66th Street, New York, N. Y.

SEMIANNUAL LIST

The Institute publishes a *Semiannual List* of all papers by members of the staff and those working under grants from the Institute, stating the title and place of publication of these reports. The *Semiannual List* will be sent regularly upon application.

SURFACE TENSION OF SERUM.

XI. An Improvement of the Technique for Measuring Surface Tension.

By P. LECOMTE du NOÜY.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

Plate 1.

(Received for publication, May 1, 1924.)

The great number of measurements of surface tension with the du Noüy tensiometer performed in this laboratory during the past 4 years[1] has progressively revealed most of the causes of error and disturbing factors which at first prevented the reproducibility and the perfect checking of the measurements. These causes are of two kinds: mechanical and chemical. They are both a consequence of the fundamental fact, pointed out in the preceding papers, that, when colloidal substances are dealt with from the standpoint of surface tension, the time factor has to be considered, since adsorption takes place in the surface layer, thus changing the value of the surface tension continually. Hence, at least two values of the surface tension were always measured: the initial value, and the value after an empirically determined time, say 2 hours. After 2 hours the surface tension is practically constant, an equilibrium having been established between the surface layer and the bulk of the solution. It has been shown that part of all the substances present in the solution were adsorbed, with the colloids, in the surface layer, and also that the slightest jarring or shaking changed the value of the surface tension by tearing the delicate film formed in the superficial layer. Very pure water and sodium chloride, and freshly cleaned glass were used. The solutions were handled in such a way that the film was not torn. This was very important, since only one measurement of every solution can be made,

[1] du Noüy, P. L., *J. Exp. Med.*, 1922, xxxv, 575, 707; xxxvi, 115, 547; 1923, xxxvii, 659; xxxviii, 87; 1924, xxxix, 37, 717; xl. 129, 133.

after it has stood for 2 hours; indeed, the tearing off of the ring breaks up the film and a second measurement gives a totally different value. In order to secure immobility of the solutions in the watch-glasses,

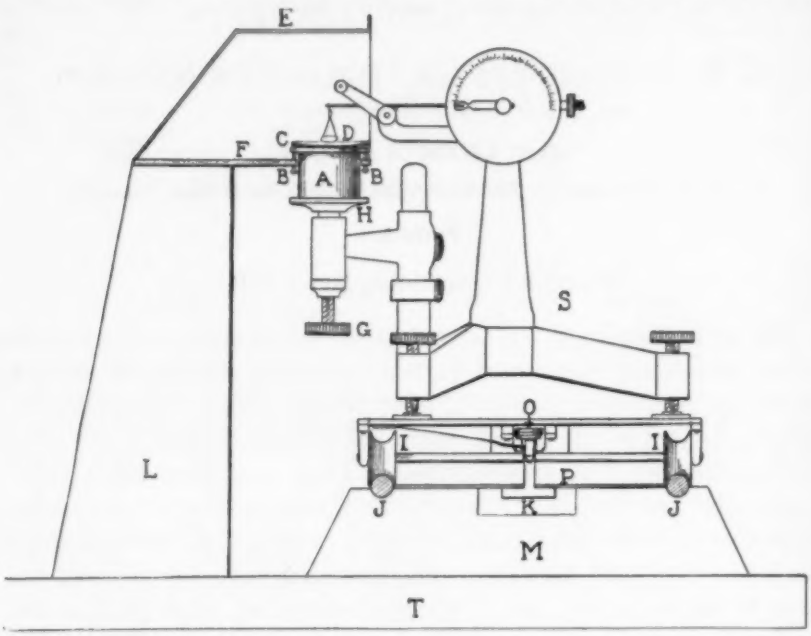


FIG. 1. Device used for making a series of measurements of surface tension without disturbing the solutions.

The tensiometer, *S*, runs on the carriage supported by the rollers, *I*, on the rails, *J*. A brake, *K*, released by the button, *O*, blocks the carriage rigidly at any given point.

A, bronze cylinder on which the watch-glass is placed. It is leveled by means of three screws, *B*, so as to be strictly parallel to the raising table, *H*. This cylinder slides in a hole made in the table, *F*. *C*, watch-glass containing the solution; *D*, platinum ring; *E*, hood protecting the solutions against dust and air currents; *F*, steel table supporting the cylinder, *A*; *G*, screw for raising table, *H*, and cylinder, *A*; *I*, rollers on which the carriage moves; *J*, rails; *K*, brake; *L*, support of table, *F*, and hood, *E*; *M*, metallic base supporting rails, *J*; *O*, button releasing brake, *K*.

and a rise of the surface so that its plane remains strictly parallel to the original plane, a metallic bench was made, and screwed on to a very substantial table. On this bench, twenty-four holes were bored,

the diameter of which was 40 mm. (Fig. 1 and Plate 1). Three leveling screws (*B*) were placed at 120° from each other around the circumference of every hole. Twenty-four hollow bronze cylinders (*A*), bearing a collar designed to rest on the leveling screws (*B*), were placed

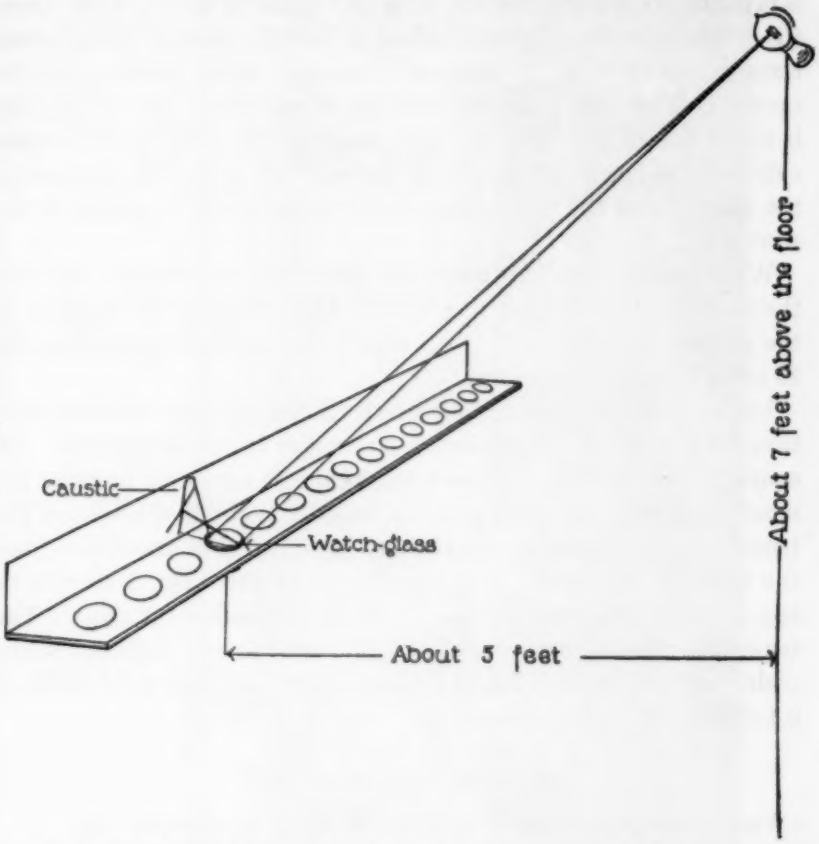


FIG. 2. Illustrates the method used in order to level the bronze cylinders.

in the holes. They could thus be leveled perfectly and raised by the moving table (*H*) of the tensiometer.

The tensiometer is placed on a small carriage rolling on two steel rails (*J*). A brake (*K*) is provided so as to block the whole moving system in a perfectly fixed position. The watch-glasses are placed on the bronze cylinders (*A*) and protected against dust and air currents by the metallic hood (*E*).

The cylinders are leveled in the following way. The watch-glass being in position, a powerful Mazda lamp is placed in such a way as to produce, by reflection on the concave face of the watch-glass, a brilliant caustic[2] on the white wall of the hood (Fig. 2). The tensiometer is brought in front of it and the table (*H*) raised by means of the screw (*G*). When the watch-glass is raised so that the plane of the solution remains parallel to its original plane, no jerky motion of the caustic is to be seen. On the contrary, if one side of the watch-glass is raised before the other, in other words, if the base of the bronze cylinder is not perfectly parallel to the table (*H*), a shift is observed in the caustic, and the parallelism can be reestablished by means of the screws.

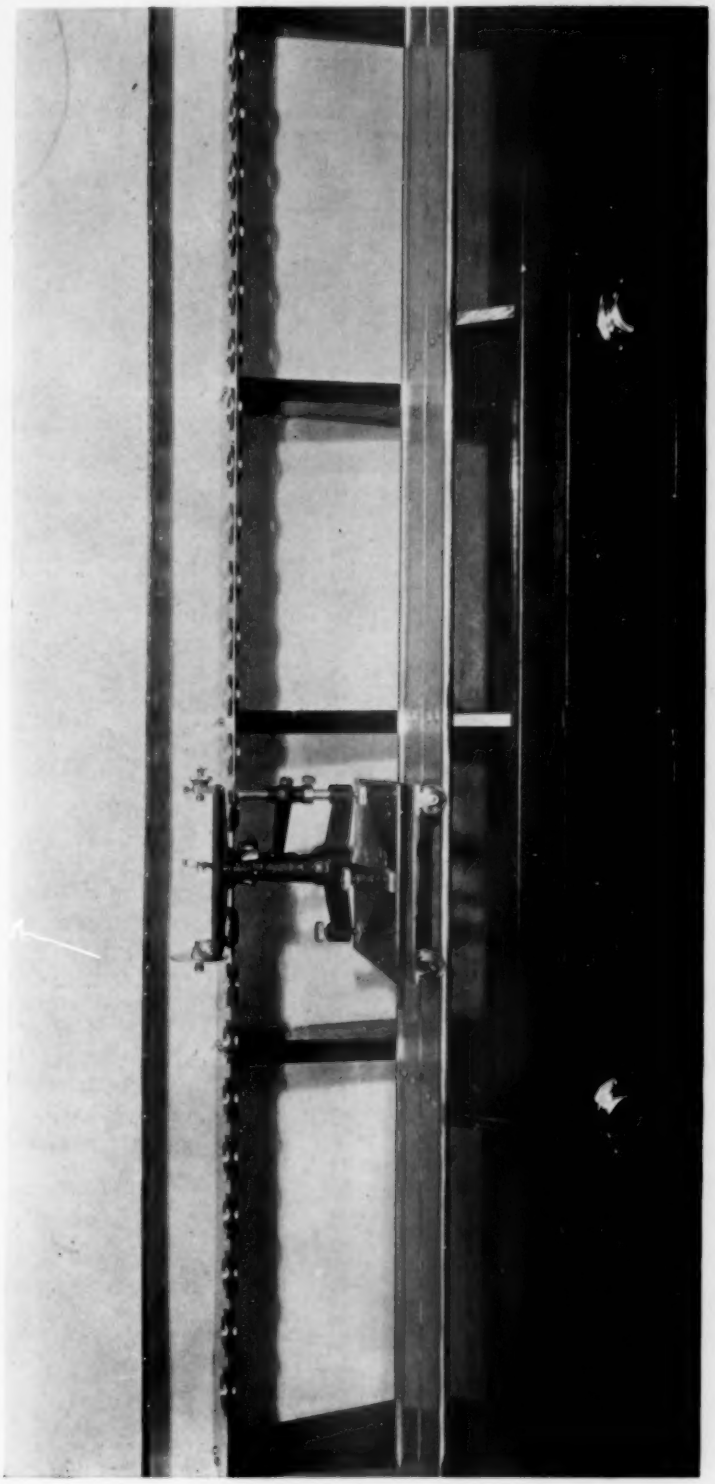
When measurements are made, the same time should elapse between the moment the platinum ring touches the surface of the liquid, and the moment it breaks off. Our experience was that approximately 15 seconds were satisfactory.

Under such conditions, the surface tension of pure colloidal solutions may be measured with as much accuracy as can be expected. Of course, when the initial values are taken, it is advisable to make the measurements as rapidly as possible, in order to obtain the value of the tension before the colloidal molecules have had time to wander from the bulk of the solution to the surface. In this case, 5 seconds or less are sufficient, and the results will check within 0.1 dyne. The tensiometer has been improved recently, and has now a direct reading scale. A vernier and a larger scale allow the readings to be made to 0.1 dyne with great ease.

EXPLANATION OF PLATE 1.

View of apparatus, described in this paper, set up for measurements.

[2] Since the reflecting surface is the watch-glass (which is a segment of a sphere) the reflected beam appears on the intercepting surface as a caustic and not as an image.



(du Noüy: Surface tension of serum. XI.)

A NOTE ON THE SIMILARITIES BETWEEN THE CURVES OF GROWTH AND OF REGENERATION.

By SAMUEL BRODY.

(*From the Department of Dairy Husbandry, University of Missouri, Columbia.*)

(Received for publication, March 24, 1924.)

In a preceding communication on the growth of the dairy cow,[1] it was shown that the course of extrauterine growth of this animal can be satisfactorily represented by the equation

$$Y = A - Be^{-kt} \qquad (1)$$

in which Y is the weight or any linear measure at the age, t, e the base of natural logarithms, k the velocity constant of growth, and A and B are constants, A having a smaller numerical value than B. Since there is such a difference between the values of A and B, the curve of equation (1) when extrapolated to include the intrauterine period of growth does not cut the time axis at zero. Instead of beginning at conception, it begins sometimes after, as illustrated in Fig. 1a, thus indicating either a relatively slow phase of growth of the fetus in the early stages or a vigorous growth, but of a type which cannot be expressed in terms of weight or linear measurements. It may also be, of course, that an error was made in the choice of the equation to represent the course of growth, that is the equation (1) may be a purely empirical one representing only a part of the course of growth. Recently the writer chanced to find a paper which promised to throw some light on this problem. Miss Durbin,[2] studying the rate of regeneration of the tail of the tadpole *Rana clamitans*, found an apparently slow phase of regeneration during the first 3 to 5 days following the removal of part of the tail, and she advanced a very plausible explanation for this apparent slow phase of regeneration.

[1] Brody, S., and Ragsdale, A. C., *J. Gen. Physiol.*, 1923–24, vi, 329.

[2] Durbin, M. L., *J. Exp. Zool.*, 1909, vii, 397.

This suggested the possibility that there may be a similar slow phase, and for the same reasons, in the beginning of growth of the fetus, and it was, therefore, thought interesting to compare the course of growth and of regeneration in order to bring out such a similarity if it exists.

Accordingly, equation (1) was fitted to the data on regeneration of the tail of the tadpole with the results shown in Fig. 1, b. A comparison between Figs. 1a and 1b shows that the agreements and the discrepancies between observed and computed values are of the same order for the curves of growth and regeneration. It appears then, that the course of growth and the course of regeneration are governed by the same laws and that the apparent slow initial phases of growth and of regeneration are due to the same causes. Miss Durbin observed the slow initial phase of regeneration and on the basis of histological studies of the regenerating tissues she explained it as due, in part at least, to the formation of a cap of embryonic cells which serves as a basis for the more active regeneration. In other words, regeneration is vigorous from the beginning, but it is principally qualitative and cannot, therefore, be fully expressed in terms of linear measurements.

If growth and regeneration are governed by the same laws as was assumed in the last paragraph, then it is natural to extend the explanation for the slow phase of regeneration to include the slow phase of growth. That is, that growth in the early stages like regeneration in the early stages is vigorous, but the growth is principally qualitative and cannot be fully represented in terms of grams or centimeters; and that if these qualitative changes at the beginning of growth or of regeneration could be converted into some common unit of growth energy with the subsequent measurable quantitative changes, the apparent initial lag (a on Fig. 1) would disappear, and with this the differences in the numerical values of A and B in equation (1) would disappear. This comparison between the curves of growth and of regeneration, therefore, leads to the tentative conclusion that the initial lag in the curves is only apparent due to our inability to convert the initial qualitative growth into quantitative units, and that equation (1) is rational potentially capable of expressing the whole course of growth and of regeneration.

Incidentally, the comparison between the curves of growth and of regeneration made in this note adds the following to our knowledge

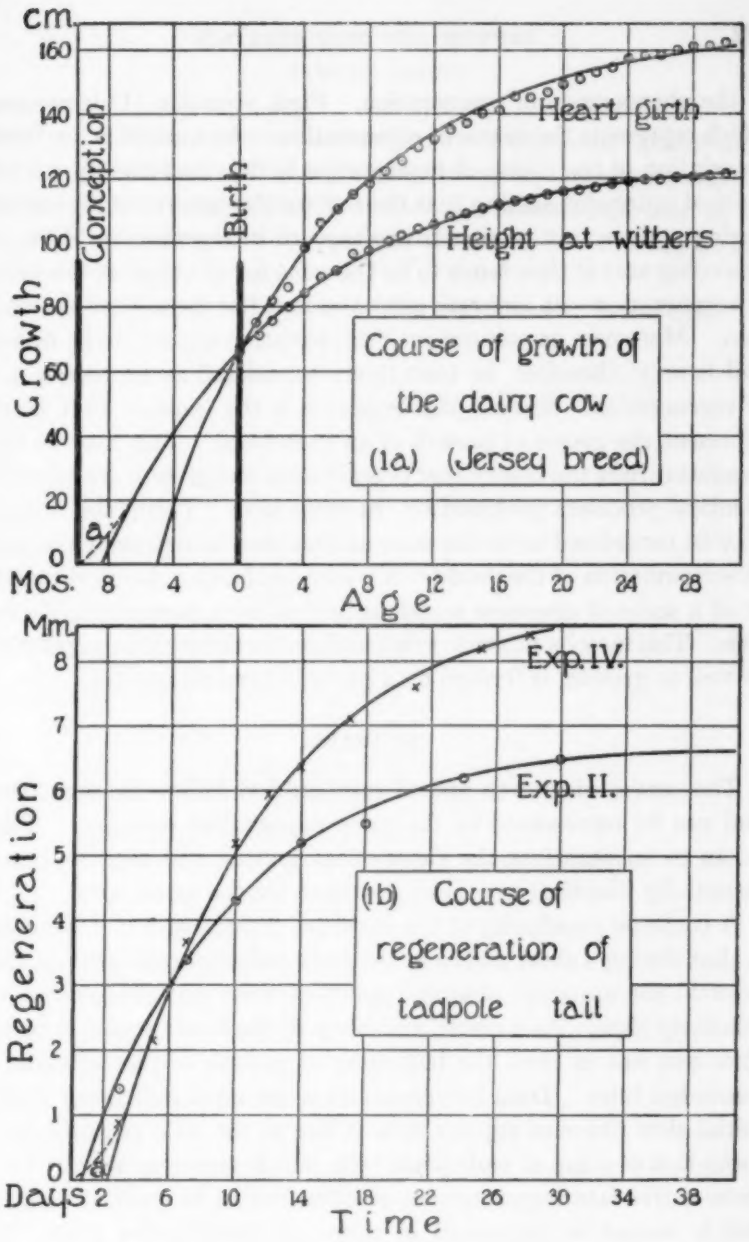


FIG. 1. The relative course of growth and of regeneration. The circles and crosses represent the observed values, the smooth curves represent equation (1) given in the text.

of the phenomenon of regeneration. First, equation (1) is presented which represents the course of regeneration. An unsatisfactory verbal description of the course of regeneration is thus replaced by a mathematical expression stating that the ratio of the amount of regeneration during a given unit of time to the amount of regeneration during the preceding unit of time tends to be the same for all stages of the process of regeneration—at any rate after the first few days after the operation. Moreover, as pointed out,[1] this equation appears to be rational, and it may, therefore, be tentatively considered as expressing a law of regeneration. Second, this equation is the same as that used to represent the course of growth of an individual. This may be taken to substantiate the theory that regeneration and growth are essentially identical processes governed by the same laws. Third, this equation may be considered to be the same as that used to represent the course of accumulation of the product of a monomolecular chemical reaction, or of a series of chemical reactions limited by a monomolecular reaction. This may be taken to substantiate the theory that regeneration as well as growth, is limited by a physicochemical process.

SUMMARY.

The curves of growth and of regeneration follow the same course, and can be represented by the same exponential equation. This is taken to substantiate the theory that growth and regeneration are essentially identical processes governed by the same laws.

A common peculiarity of the curves of growth and of regeneration is that during a short period in the early stages of regeneration and of growth, the apparent observed speed of these processes seems to be relatively slow. As a result, the curve of the fitted equation cuts the time axis not at zero, the beginning of growth or regeneration, but somewhat later. Data on regeneration are cited indicating that the initial slow phase of regeneration is due to the time required for the formation of a cap of embryonic cells which serves as a basis for the more active later regeneration; in other words, to qualitative growth which cannot be expressed in terms of quantitative units. It is suggested that the apparent initial slow phase of growth of the individual from the fertilized egg is due to a similar qualitative growth. It is suggested that if the initial qualitative changes could be con-

verted into some common unit with the subsequent quantitative changes, the apparent initial lag would disappear, and the exponential equation representing the course of these processes would then be the same as the equation used to represent the course of a monomolecular chemical reaction.

Certain implications of this reasoning are discussed in the text.

THE EFFECT ON THE EXTERNAL PARATHYROID GLANDS OF THE EXPOSURE OF RABBITS TO ULTRA-VIOLET LIGHT.

By J. H. B. GRANT and FREDERICK L. GATES.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

PLATE 2.

(Received for publication, April 2, 1924.)

Because many instances have been observed of the effects of light on metabolic processes, and because in the higher animals the glands of internal secretion act in part as chemical regulators of metabolism, we are undertaking certain studies on the effects on the endocrine tissues of exposure of the skin to ultra-violet light. In preparation for these studies it seemed desirable to make preliminary observations on the reactions of all the ductless glands available for examination, and to note such changes in weight and microscopic appearance as might indicate abnormal activity. Such a study is the more necessary because of various interrelationships among the endocrine glands that make isolated observations on a single tissue of doubtful value.

Dr. Wade H. Brown and Dr. Louise Pearce have directed attention to this interdependence among certain of the glands of internal secretion in connection with their extensive studies on experimental syphilis and carcinoma in the rabbit. During the past 2 years they have collected data on individual and seasonal variations in the size, appearance, and functional activity of the endocrine tissues of a large number of normal animals.[1] These valuable records, mostly unpublished, they have kindly placed at our disposal. It would have been fruitless to make the observations on which this paper is based without the extensive control material thus afforded us. Even during the period covered by the experiments—November to February—there occurred in certain of the endocrine tissues a change, regarded as

[1] Brown, W. H., Pearce, L., and Van Allen, C. M., *Proc. Soc. Exp. Biol. and Med.*, 1923–24, xxi, 373.

seasonal, of as much as one-fourth in their weight. Individual differences were even greater. It was essential, therefore, to establish a concurrent base line with a considerable number of normal rabbits in order to determine those differences from the normal that could be ascribed to the experimental procedures. This explains why the rabbit was chosen for our study.

A summary of the results of this preliminary study of the effects of ultra-violet light on normal rabbits has already been published.[2] Among the changes found in various endocrine glands, those involving the external parathyroids arrested our attention and seem worthy of more extended consideration. The present paper deals mainly with these secondary effects of ultra-violet light on the parathyroid glands and only a brief reference will be made to the observations on the other glands of internal secretion in order to correct and extend the earlier data in the light of subsequent observations.

Experimental Procedure.

Experiment 1.—Albino or light colored adult male rabbits weighing more than 1,600 gm. were selected from healthy stock and caged separately in a well lighted room under the usual conditions of ventilation and diet maintained for normal animals. Ears were shaved and backs closely clipped before exposure and at intervals during the experiment. In groups of six these rabbits were exposed for 30 minutes daily over periods of 3 to 7 weeks to the unfiltered radiations of a quartz mercury arc lamp (110 volts, 4 amperes) at a distance of 1 meter. This arbitrary exposure caused at first a marked hyperemia of the exposed skin followed by a persisting erythema and some desquamation, especially of the ears. The erythema was followed by a leathery thickening of the skin, notably of the back, and moderate pigmentation. During the first few days certain of the rabbits were drowsy, refused some of their food, and lost weight. The feces were oft n soft and three animals had a transient diarrhea. One of these three died of und termined cause on the 5th day of exposure. The other rabbits soon regained their normal weight and appetite and appeared to be in good condition throughout the experimental period. Two rabbits, one showing a severe coccidiosis, the other a carcinoma of the liver with metastases, were discarded at autopsy. Th dose chosen, while somewhat toxic at first, apparently was relatively ineffective during the final periods when the thickening and pigmentation of the skin had occurred.

At weekly intervals from the 3rd to the 7th week, five or six of the exposed

[2] Grant, J. H. B., and Gates, F. L., *Proc. Soc. Exp. Biol. and Med.*, 1923–24, xxi, 230.

rabbits and two normal controls were etherized and bled from the aorta. The following glands were then weighed and examined in gross and microscopically: pineal, hypophysis, external parathyroids, thyroid, thymus, adrenals, and testicles. The weights of these glands were recorded in mg. per kilo net body weight after removal of the gastrointestinal tract and its contents and the expression of bladder urine.

We had, in addition to the two controls for each group of rabbits, the observations of Dr. Pearce and Dr. Van Allen on groups of twelve to twenty-four normal animals examined within 2 or 3 weeks of the time our rabbits were killed. It was found necessary thus to confine the control groups to normal rabbits killed almost coincidently because of the apparently seasonal variations in the ratio gland : body weight during the course of the experiment. The changes in the endocrine glands, in weight per cent, compared with the figures for the normal

TABLE I.

Effect of Daily Exposure of Normal Rabbits to Ultra-Violet Light on the Weights of Certain Endocrine Tissues.

Glands.	Per cent change from normal						
	Time of exposure in weeks.						
	1	2	3	4	5	6	7
Pineal	±0	+24	+11	−5	+9	+7	+18
Hypophysis	+16	+25	+19	+14	+17	+7	+8
External parathyroids	+10	+41	+39	+56	+26	+36	+18
Thyroid	+10	+10	−17	−20	−2	−11	−12
Thymus	+20	−12	+13	+5	+9	−14	+16
Adrenals	+8	−17	−3	+3	+17	+19	+10
Testicles	+1	−14	+18	+4	+36	−3	+2

animals, are shown in Table I. The figures in this table differ somewhat from those already published. The former figures were based on an average of the tio gland : body weight of all the normal animals examined during the course of the experiment. A review of these data showed that a closer approximation to the actual ratios would be obtained by comparing the glands of radiated animals only with those of normal rabbits examined during the month in which the radiated animals were killed. This has been done in Table I.

When the results of 3 to 7 weeks daily exposure to the quartz mercury arc lamp had been obtained and changes noted in some of the glands after 3 weeks radiation, other groups of rabbits were radiated in an identical manner for 1 and 2 weeks to fill the gap in the earlier observations. The resulting figures have been included in Table I, but they are not strictly comparable with those obtained earlier because they are based on February controls, whereas the earlier experiments were com-

pleted when the normal control figures for several of the glands differed considerably from those obtained in February. The high figures for the thyroid and adrenal glands after 1 week of exposure may be due to marked congestion and even gross hemorrhages sometimes found in these glands at autopsy.

The Secondary Effects of Ultra-Violet Light on the Parathyroid Glands.

Without a discussion of the possible significance of the changes in the other endocrine tissues, attention may be directed to the parathyroid glands. Only the two external parathyroids were considered in this study. The internal glands, buried in thyroid tissue, are inaccessible to direct determination by weight and their size must be estimated by the indirect method employed by Luce.[3] Hence our figures are relative rather than absolute. It seems improbable, however, that exposure of an animal to ultra-violet light would have diverse effects on the external and the internal glands, and we have assumed the changes in the external parathyroids to be an index of the secondary effects of light on the whole parathyroid apparatus. If this is the case, it appears that exposure of the skin to the radiations of a quartz mercury arc lamp caused a marked increase in the weight of the parathyroid glands. This increase began during the first few days of exposure. It was the more striking in that it occurred during a period when the parathyroids were normally undergoing an apparently seasonal diminution in weight. The average weights of the two external parathyroid glands in the normal rabbits decreased at a fairly uniform rate from 6.9 mg. per kilo net body weight in November to 5.4 mg. in February.[4] In the radiated rabbits not only was this normal decrease arrested but the external glands underwent an active hypertrophy. The peak in the size of the external parathyroids occurred after 4 weeks daily exposure to the quartz lamp. Then the average weight of the two glands was 10 mg. per kilo body weight, an increase of 56 per cent over the normal weight for December, when these rabbits were examined. Fig. 1 is designed to show the relation of the changes in weight of the external parathyroids of radiated

[3] Luce, E. M., *J. Path. and Bact.*, 1923, xxvi, 200.

[4] This gradual decrease in the normal size of the parathyroids from November to February may be significant in relation to the seasonal incidence of such diseases as rickets and tetany and to the distribution of sunlight through the year.

rabbits to the changes occurring in normal animals during the same season of the year.

The probable errors in the calculation of the weights of the parathyroid glands in the normal rabbits and in each experimental group have been calculated by Peter's approximation formula and recorded in the chart. The chances are even that the correct values for each determination lie within the limits defined by the probable error.

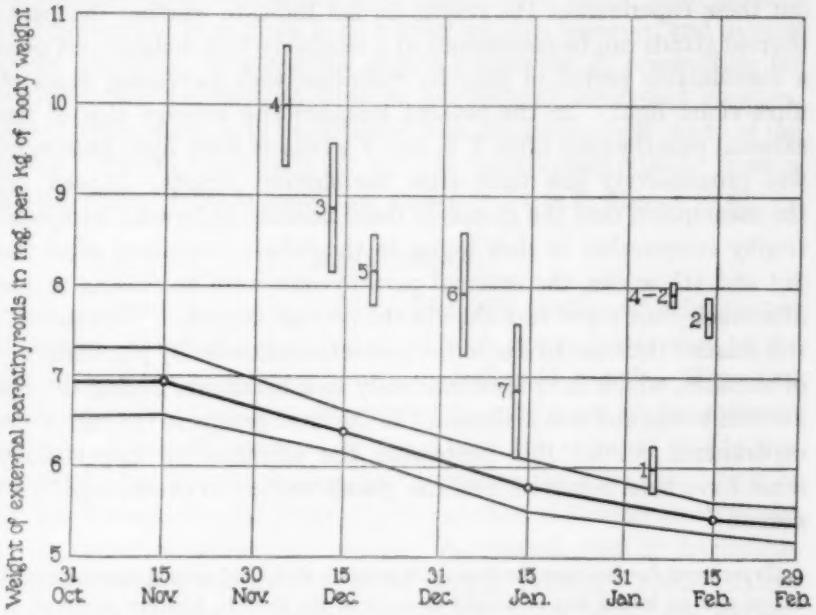


FIG. 1. The average weight of the external parathyroid glands in normal and radiated rabbits.

Solid line with circles. Weight of glands of normal rabbits examined during each month grouped on the 15th of the month. The corresponding lines above and below the normal indicate the *probable error* in these determinations.

Vertical rectangles. Average weights of glands and *probable error* for each group of radiated rabbits. Figures indicate weeks of daily exposure.

4–2 refers to Experiment 2.

Fig. 1 is based on the average figures for all the rabbits in each group. The probable errors indicate that wide individual differences were found in the ratio gland : body weight of the animals in both the normal and the radiated groups. There was, indeed, some overlapping of the groups, so that some of the normal

rabbits had larger parathyroid glands than certain of the radiated animals. This fact may be interpreted in either of two ways. It is possible that some of the radiated rabbits did not react to the light treatment by parathyroid hypertrophy. On the other hand, the smallest glands found in normal animals weighed 40 per cent less than the smallest glands found in radiated rabbits. The parathyroid glands in these radiated rabbits, though small, may nevertheless have undergone a considerable hypertrophy.

On account of the arbitrary dosage (30 minutes daily) used throughout these experiments, the results do not indicate whether the parathyroid glands can be maintained at a relatively high weight level over a considerable period of time by radiation with increasing doses of ultra-violet light. In the present instance the average size of the external parathyroids after 5, 6, and 7 weeks of daily light treatment was progressively less than after the shorter periods. Indeed, on the assumption that the glands in these animals underwent an hypertrophy comparable to that found in the rabbits examined after the 3rd and 4th weeks, the external parathyroids were decreasing in size at a much more rapid rate than in the normal controls. We attribute this relative decrease in size to the protection afforded by pigmentation of the skin, which developed gradually to a maximum during the 3rd and 4th weeks and was maintained in the later groups to the end of the experiment. Under this protection the stimulus to hypertrophy must have been removed, and the glands tended to revert rapidly to normal.

Experiment 2.—Because the dose of 30 minutes daily had caused signs of intoxication and an initial loss of weight in some of the rabbits, another group of six animals was exposed to the quartz lamp for 10 minutes on the 1st day and the exposure was increased 2.5 minutes each day for a period of 4 weeks. These rabbits also exhibited some degree of erythema but showed no toxic symptoms or loss in weight. At autopsy the average weight of their external parathyroid glands was 7.92 mg. per kilo net body weight, an increase of 45 per cent over the average normal weight for February. In the preliminary report[1] this increase is given as 32 per cent. At that time the February control statistics had not been completed and this figure was based on the average normal weight for January.

Microscopic Examinations.

The external parathyroid glands from some of the rabbits in each group were fixed in Zenker's solution and sectioned for microscopic

study. These sections showed that the increases in weight were due to a true hyperplasia of the parenchymatous tissue. There was no appreciable hyperemia, edema, or fibrosis. The mass of the hyperplastic glands was made up of closely packed cells and cell columns, with large oval or rounded nuclei and finely granular cytoplasm, like those in the normal glands. Occasional mitotic figures were found in some sections of the hyperplastic tissue. In other sections some pycnosis and some vacuolation of cytoplasm were seen. Such changes were also found in sections from normal rabbits, however, and in view of the inevitable handling the glands underwent in preparation for weighing, we feel that no definite evidence of structural changes, except hyperplasia, is to be obtained from the microscopic study of the glands. Photomicrographs of external parathyroid glands from a normal and a radiated rabbit are shown in Plate 2, Figs. 1 to 4.

Blood Calcium.

The study of the effects of ultra-violet light on the endocrine tissues of normal rabbits was undertaken without any preconception of the probable effect of light upon the parathyroid glands. Hence no preliminary blood calcium determinations were made on the radiated rabbits. When the increase in the size of the external glands was observed after 3 and 4 weeks daily exposure, the calcium content of the blood serum was determined by the method of Kramer and Tisdall[5] on the rabbits of subsequent groups. As controls, similar determinations were made on the sera of 60 normal rabbits. These control determinations gave an average value of 11.9 mg. of Ca per 100 ml. of serum. The limits of variation among the normal rabbits were wide, namely 14.1 and 9.4 mg. The average calcium content of the serum of the radiated rabbits in the 5th, 6th, and 7th week groups was 12.7; 12.9; and 12.6 mg. per 100 ml. of serum.

Repeated blood calcium determinations on the rabbits of subsequent groups and on normal rabbits caged and fed under similar conditions have since led us to question the extent to which these relatively slight increases in the serum calcium of radiated animals can be attributed to the hypertrophy of the parathyroid glands. The

[5] Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, xlvii, 475.

60 determinations on normal rabbits which make up the control average of 11.9 mg. Ca per 100 ml. of serum were made mostly on animals from different sources recently put in stock, whereas the radiated animals had been caged for 5 to 7 weeks before the serum calcium was determined. Now it appears that the serum calcium of normal rabbits rises during the first weeks of caging to figures comparable to those for the radiated animals. This is shown in Table II in which the results of serum calcium determinations on Groups 1

TABLE II.

Comparison of Serum-Calcium Determinations in Normal and Radiated Rabbits.

	Mg. of Ca per 100 ml. of serum.				
	Before radiation.	Time in weeks.			
		1	2	3	4
Normal control rabbits	11.9	12.8	13.2	13.1	12.2
Radiated rabbits, Groups 1 and 2, Experiment 1; and Experiment 2	11.9	13.6	13.2	13.5	12.9

and 2 of Experiment 1, before and after radiation, have been combined with those of successive weekly determinations on the rabbits of Experiment 2, and these figures compared with similar figures for the control rabbits of these groups.

Emphasis should be placed rather on the fact that a parathyroid hypertrophy of 20 to 50 per cent does not result in a corresponding increase in the calcium content of the blood when the calcium concentration is already at or near the normal level.

DISCUSSION.

So far as we are aware parathyroid hyperplasia has been demonstrated hitherto only in clinical conditions characterized by a low blood calcium level in which it might be regarded as an effort at compensation due to physiological need. Thus Luce[5] found a true hyperplasia of the secreting cells of the parathyroid glands in rats on a low calcium diet. Erdheim[6] in rats, and more recently Ritter,[7] and

[6] Erdheim, J., *Sitzunsber. k. Akad. Wissensch. Math.-naturw. Cl. Wien*, 1914, xv.

[7] Ritter, C., *Frankf. Z. Path.*, 1920, xxiv, 137.

Pappenheimer and Minor,[8] in children, observed a hyperplasia of the parathyroids in clinical conditions diagnosed as rickets. Vines[9] refers to a similar hyperplasia in certain cases of chronic nephritis with disturbances of the calcium balance in the blood, but notes that hyperparathyroidism is not known as a clinical entity.

In the absence of a complete understanding of parathyroid function it might be difficult to demonstrate clinical changes attributable to hyperparathyroidism. Our animals showed no variations from normal that could be so interpreted. In an effort to determine the significance of the hyperplasia produced by ultra-violet light we are therefore thrown back on present day knowledge of the relation of the parathyroid glands to calcium metabolism.

The drop in blood calcium that occurs after parathyroidectomy[10–16] indicates that the maintenance of the normal calcium level is dependent upon the integrity of the parathyroid function. As is the case with other essential tissues, the normal glands appear to provide the body with a wide margin of safety in this respect so that much of the parathyroid apparatus may be removed without seriously affecting the calcium level. Nevertheless it has been amply demonstrated that in the absence of sufficient parathyroid tissue the calcium level of the blood becomes permanently lowered and can be restored only temporarily by the introduction of calcium salts.[16] It does not follow that the converse must hold true; that an excess of parathyroid tissue must cause a rise in the calcium level above normal. Other factors, unrelated to the parathyroid function so far as our present knowledge goes, seem to define the upper limit of blood calcium concentration. The normal level is at, or near, the saturation point of the serum for calcium. Howland and Kramer,[17] from a review of the literature and

[8] Pappenheimer, A. M., and Minor, J., *J. Med. Research*, 1920–21, xlii, 391.
[9] Vines, H. W. C., The parathyroid glands in relation to disease, London, 1924.
[10] MacCallum, W. G., and Voegtlin, C., *J. Exp. Med.*, 1909, xi, 118.
[11] MacCallum, W. G., and Vogel, K. M., *J. Exp. Med.*, 1913, xviii, 618.
[12] Howland, J., and Marriott, W. McK., *Quart. J. Med.*, 1917–18, xi, 289.
[13] Hastings, A. B., and Murray, H. A., Jr., *J. Biol. Chem.*, 1921, xlvi, 233.
[14] Gross, E. G., and Underhill, F. P., *J. Biol. Chem.*, 1922, liv, 105.
[15] Salvesen, H. A., *J. Biol. Chem.*, 1923, lvi, 443.
[16] Salvesen, H. A., *Acta med. Scand.*, 1923, suppl. 6.
[17] Howland, J., and Kramer, B., *Monatschr. Kinderheilk.*, 1923, xxv, 279.

their own experiments, point out that the blood serum is normally a supersaturated solution of calcium bicarbonate and secondary calcium orthophosphate. The excess of calcium presumably is held in combination with protein. Salvesen and Linder[18] refer to this calcium store as a " . . . . reserve which will furnish a new supply of ionized calcium, if this important part of the blood calcium is diminished."

If this is so, the normal parathyroid function would seem, directly or indirectly, to maintain the calcium concentration of the blood at an upper limit determined by other, independent factors. Whereas in the absence of sufficient parathyroid tissue this limit cannot be attained, even under parathyroid hypertrophy it cannot be surpassed. The effects of ultra-violet light in our normal rabbits may, therefore, have been merely to increase the factor of safety of the parathyroid glands without producing any significant change in the blood calcium level. Experiments are in progress which may throw light upon this question.

The mechanism by which the parathyroid glands maintain the blood calcium level is not yet understood. Salvesen[16] found that the transient character of the rise in blood calcium caused by calcium injections in parathyroidectomized dogs was due to a rapid elimination of the injected calcium, mainly through the intestine. His observations, therefore, suggest that the parathyroid glands influence the passage of calcium through the intestinal wall.

A diminished absorption or an increased excretion of calcium *via* the intestine has been observed also in infantile tetany and certain cases of rickets in which low blood calcium values are found. This fact, in association with the well recognized effects of ultra-violet light in the treatment of rickets and tetany led Orr, Holt, Wilkins, and Boone[19] to ascertain whether ultra-violet light therapy promoted the absorption of calcium and phosphorus from the intestines. They found that in infants with active rickets there was little if any retention of calcium and phosphorus in the body and that most of the ingested phosphorus and almost all of the calcium was to be found in the stools.

[18] Salvesen, H. A., and Linder, G. C., *J. Biol. Chem.*, 1923, lviii, 635.

[19] Orr, W. J., Holt, L. E., Jr. Wilkins, L., and Boone, F. H., *Am. J. Dis. Child.*, 1923, xxvi, 362.

After treatment with ultra-violet light, however, coincident with the healing of the rachitic lesions there occurred a greatly increased retention of calcium and phosphorus, so that one-third to one-half of these elements was retained.

So little is known about the factors controlling phosphorus metabolism that we prefer not to include this element in this discussion. But our experiments, which demonstrate a striking secondary effect of exposure to ultra-violet light upon the parathyroid glands, considered in relation to those of Salvesen, and of Orr, Holt, Wilkins, and Boone, may provide another link in the chain of evidence to explain the effects of ultra-violet light therapy on calcium metabolism in rickets and tetany. It seems not improbable that the parathyroid glands are directly concerned in the process; that one result of exposure of the skin to ultra-violet light is some alteration in the composition of the blood to which the parathyroids are sensitive, and to which they react by hypertrophy. If this hypertrophy is accompanied by an increase in functional activity it should tend to counteract those processes which result from parathyroid dysfunction, among them the failure of the intestines in regard to the absorption and retention of calcium, with a consequent low calcium level in the blood.

SUMMARY.

A preliminary study has been made of the effects of radiation of normal rabbits with ultra-violet light upon the weight and microscopic appearance of certain of the endocrine tissues.

Among these tissues the external parathyroid glands showed a consistent reaction. The exposures caused an hypertrophy of the glands amounting in some instances to more than half the normal weight. The hypertrophy was a true hyperplasia of the parenchymatous tissue. It was not accompanied by any significant increase in the blood calcium above the normal level.

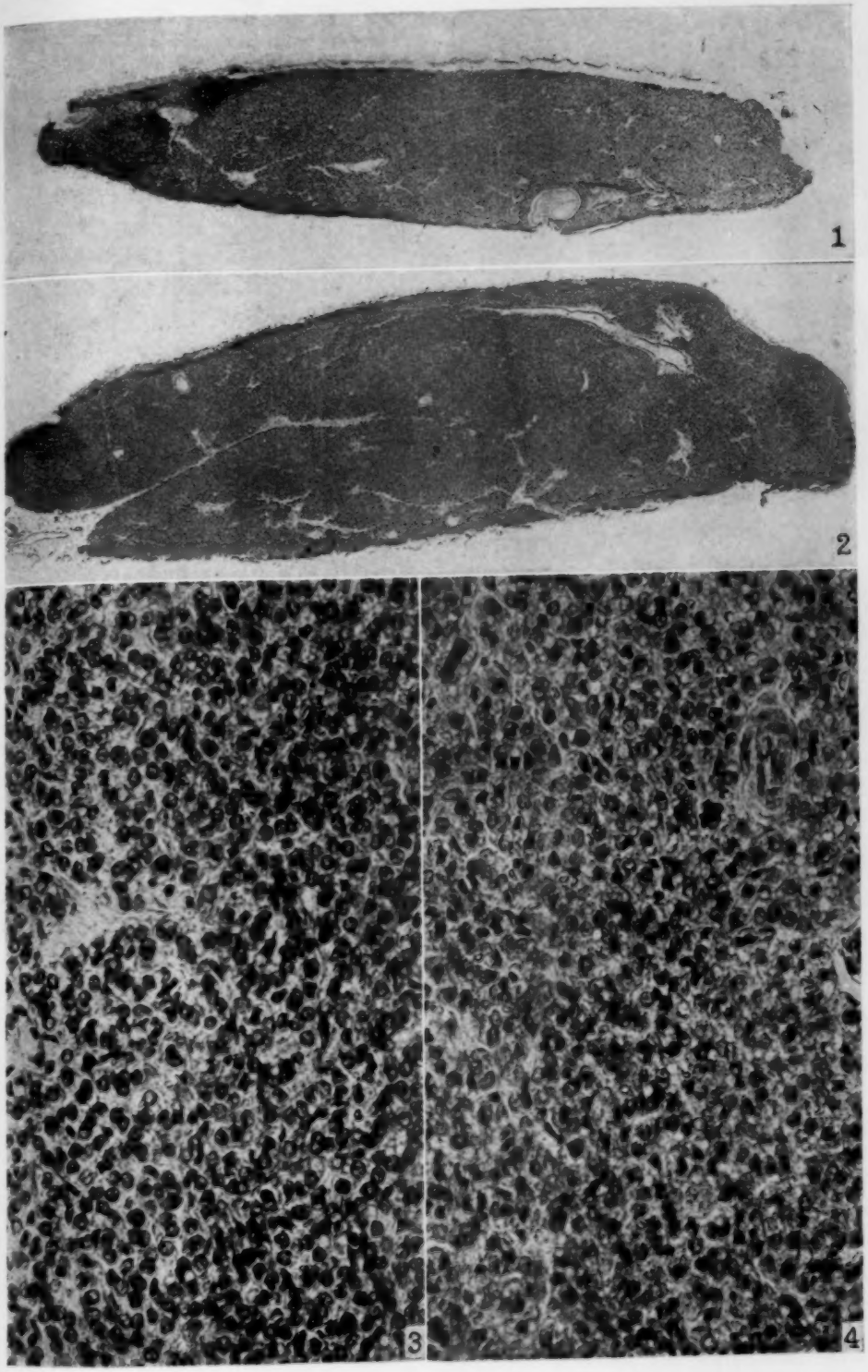
EXPLANATION OF PLATE 2.

FIG. 1. Section of external parathyroid gland of normal rabbit. × 26.

FIG. 2. Section of external parathyroid gland of rabbit radiated daily for 3 weeks with ultra-violet light. × 26.

FIG. 3. Higher magnification of section shown in Fig. 1. × 362.

FIG. 4. Higher magnification of section shown in Fig. 2. × 362.



(Grant and Gates: Effect on parathyroid of ultra-violet light.

WAVE LENGTH OF LIGHT AND PHOTIC INHIBITION OF STEREOTROPISM IN TENEBRIO LARVÆ.

By W. J. CROZIER.*

(*From the Zoological Laboratory, Rutgers College, New Brunswick.*)

(Received for publication, April 17, 1924.)

I.

The positive stereotropism of quietly creeping larvæ of the beetle *Tenebrio* may be suppressed by light of sufficient intensity (1). That stereotropism may inhibit negative phototropism in these larvæ was observed by Loeb (2). Horizontal light of low intensity incident upon the larva through a vertical glass plate in lateral contact with which the animal is creeping, does not modify the stereotropic behavior while in full contact with the glass, nor the stereotropic orientation of the anterior end (1) upon its extension beyond the limit of the plate. Such light is, however, adequate to enforce negative phototropism in the absence of unilateral contact with a surface. Light of sufficiently great intensity induces prompt photic orientation, involving movement away from the contact surface. There is a fairly sharp value of the light intensity such that a slightly lower value fails to inhibit stereotropism while a slightly higher value almost always does inhibit it. By repeated trials with a single individual it is found that a certain intensity of light may fail to induce photic orientation away from the contact plate, but will nevertheless just inhibit stereotropic bending of the anterior end of the larva when it has passed the plate. The distinction while real is a slight one and not easily measurable, because the expression of the tendency to bend the anterior portion of the body varies with rate of creeping.

The results of attempting to balance stereotropism and phototropism are the same if the stereotropic creeping is begun under weak red light and then the horizontal white light admitted, or if the creep-

* Research Associate, Carnegie Institution of Washington.

ing is begun under the influence of the white light and the contact established secondarily. In the present series of experiments no attempt was made to analyze stereotropism during reversed creeping (1).

In measuring the "photic equivalent of stereotropism" the distances from a source of white light were found by trial at which (*a*) the light was almost always sufficient to enforce orientation away from a vertical piece of plate glass, and (*b*) at which the light just failed to do this. These distances, in ten series of experiments involving numbers of larvæ in each, were found to differ by at most some 4.0 per cent of the mean (or "critical") distance. A result with about the same degree of uncertainty is obtained by averaging the "critical distances" observed with different individual larvæ. In an earlier experiment, with temperature 16°C., the critical intensity was 132 m.c. (1). Later trials, at 22°–23°C., but with a different lamp, gave 136 m.c. as the mean intensity for inhibition of stereotropism.

II.

Measurements of the relations between wave length of light and stimulating power are less easy to obtain in connection with phototropic movements than in "reflex" responses determined by changing the light intensity. This is due to the fact that unless circus movements are studied, it is difficult to obtain a precise measure of the orientation effect in terms of constant action of the light. In a subsequent paper a case of this type will be presented (3). The behavior of *Tenebrio* larvæ, however, permits determinations of the intensities of light of given wave lengths required to produce a definite effect, namely the phototropic suppression of stereotropic response. It is to be taken for granted that the same photosensory effect, regardless of the value of the incident wave lengths, will be just sufficient to produce central nervous inhibition of the impulses derived from lateral contact. The experiment thus becomes strictly comparable with that performed by Hecht (4) with the reaction time of the siphon response of *Mya* in colored lights.

As source of light a 400 watt tungsten condensed filament was employed in conjunction with Wratten Light Filters 70, 71A, 72, 73,

CORRECTION.

On page 649, Vol. vi, No. 6, July 20, 1924, in Table I, in the heading for the fourth column, for *Relative critical energy, lamp and filter* read *Relative energy, lamp and filter*.

In the same column, the last figure, for *381.0* read *38.1*.

74, 75, 76. The method whereby these approximately monochromatic filters are used to obtain light of known intensity is described by Hecht (4). The percentage transmission being given for each filter (5) at intervals of 10$\mu\mu$ wave length, and the emission curve of the lamp known, there is obtained the total relative energy transmitted by each filter. These figures, together with the distances from the source just sufficient to give light enough to produce negative phototropism in spite of an inhibiting lateral contact, permit calculation of the "critical" photic energy for each filter.

The result of determinations made at 22°–23°C. with larvæ of uniform size, not recently emerged from ecdysis, and previously dark adapted for some hours, is given in Table I. I am indebted to Mr.

TABLE I.

No. of filter.	Modal wave length.	Critical distance.	Relative critical energy, lamp and filter.	Reciprocal of relative critical energy, as per cent of maximum.
	$\mu\mu$	cm.		
70	680	31.0	366.8	0.355
71A	653	59.0	413.3	1.14
72	612	61.5	34.8	1.47
73	578	148.0	88.6	33.4
74	532	165.0	36.8	100.0
75	492	87.0	106.9	9.66
76	450	94.8	381.0	3.20

R. Forer for assistance with the experiments. The measurements of critical distance, obtained as previously described, are subject to an uncertainty of between 1.6 and 4.0 per cent in the several cases. This amount is quite negligible in view of the large differences associated with the differences of wave length.

III.

The reciprocals of the relative energy required with each filter to just suppress stereotropism may be regarded as giving the absorption spectrum of the photoreceptive system (4). Presumably, an essential property of the photosensitive material involved in producing photic stimulation is described by the curve relating efficiency in stimulation to wave length (4, 6, 7). This cannot be fully as-

sumed, however, without disregarding absorption by the skin of the animal and by the accessory receptor structures. As measure of efficiency in stimulation, the reciprocal of critical relative energy, with that of Filter 74 as 100, is plotted in Fig. 1.

A sharp maximum of stimulating efficiency lies in the region 530 to 540$\mu\mu$. On either side of this maximum the relative stimulating

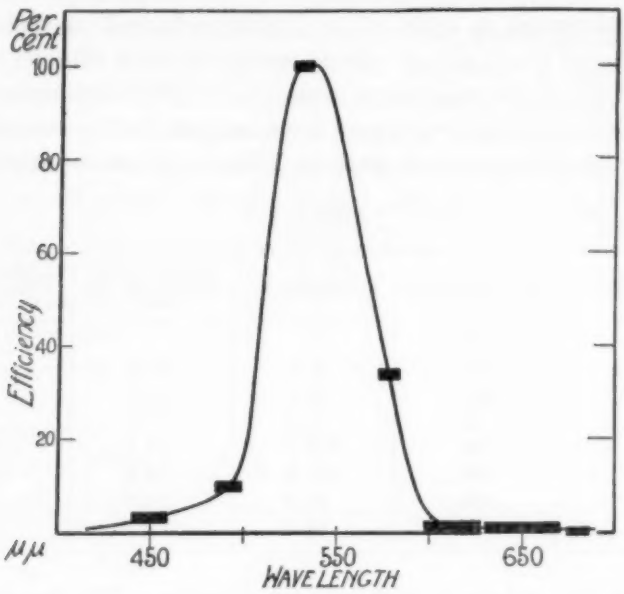


FIG. 1. Reciprocals of the relative critical energies in different regions of the spectrum required to counterbalance positive stereotropism in *Tenebrio* larvæ are plotted as percentages of the maximum effect. Each determination is given as a bar of such length as to include three-quarters of the radiation transmitted by the corresponding filter. The curve shows the distribution of the absorption effective in stimulation.

power quickly declines. This is made evident at once by the fact that the critical distance with the unscreened lamp was 169.4 cm., while with Filter 74 interposed this distance was reduced only to 165 cm. Practically the whole of the stimulation is due to yellow-green light. The numerical value of the stimulating power in other spectral regions is lower in proportion to the lesser effective absorption of such radiations by the photoreceptive system.

The larvæ of *Tenebrio* agree with certain other forms in showing a maximum sensitivity in the yellow-green. The shape of the stimulation curve (Fig. 1) differs from that described for *Mya* (4), where the maximum is at about 500$\mu\mu$, in showing a smooth absorption maximum devoid of prominent asymmetrical distortion. In this it resembles what may be assumed to be the usual condition in species which have been studied by the "equal energy spectrum" method (8). The region of maximum efficiency is not the same, however, as in the blow-fly larva, where the peak is at about 505$\mu\mu$ (8, *b*). The maximum obtained for *Tenebrio* lies at approximately 535$\mu\mu$, close to that given for certain flagellates, as *Spondylomorum* (8, *b*). The attempt by this "absorption maximum" procedure to discover if forms taxonomically related possess the same photosensitive substances, even with the supposition that the photochemical system is simple, is thus confronted by serious difficulties. The wave length of maximum effectiveness may apparently be different in different developmental stages of the same arthropod, and in the same species may vary according to the character of the optic pigmentation (9). Perhaps a chief obstacle lies in the unknown share of the skin and accessory structures in absorption. Thus for *Holothuria captiva* sunlight passed through an extracted solution of the green integumentary pigment is as effective for stimulation of the integument as unfiltered sunlight (10); one is unable to tell if light absorbed by the green pigment in this case (that is, wave lengths shorter than 540$\mu\mu$) could or could not be especially effective in stimulation. The ocelli of *Tenebrio*, probably sole receptors in phototropism lie beneath the brownish chitinous investment of the head. Direct observation shows that this chitin absorbs red rays (beyond 660) very slightly, but that transmission is distinctly reduced below 510, thus possibly accounting for the abrupt decline of the left-hand side of the curve in Fig. 1.

SUMMARY.

A definite intensity of white light is required (about 136 m.c.) to produce negative phototropic orientation of creeping *Tenebrio* larvæ away from contact with a vertical glass surface. This gives a measure of stereotropism in terms of phototropism, or reciprocally.

The effectiveness of light for the suppression of stereotropism varies with wave length. It is therefore simple to obtain a measure of the relation between wave length and stimulating efficiency in this case of phototropic orientation.

By determinations of the minimal energy required to inhibit stereotropism with different regions of the spectrum, it is found that the maximum effectiveness is sharply localized in the neighborhood of 535$\mu\mu$.

The curve connecting stimulating efficiency with wave length, while giving a picture of the effective absorption by the photosensory receptors, probably does not permit accurate characterization of the essential photosensitive material.

BIBLIOGRAPHY.

1. Crozier, W. J., *J. Gen. Physiol.*, 1923–24, vi, 531.
2. Loeb, J., Untersuchungen zur physiologischen Morphologie der Tiere. 1 Ueber Heteromorphose, Würzburg, 1891.
3. Crozier, W. J., and Cole, W. H., *Anat. Rec.*, 1923, xxvi, 362.
4. Hecht, S., *J. Gen. Physiol.*, 1920–21, iii, 375.
5. Wratten light filters, Rochester, 5th edition, 1922.
6. Lasareff, P., *Ann. Physik*, 1907, xxiv, 661.
7. Hecht, S., and Williams, R. E., *J. Gen. Physiol.*, 1922–23, v, 1.
8. Loeb, J., and Wasteneys, H., *J. Exp. Zool.*, 1916, *a*, xx, 217. Mast, S. O., *J. Exp. Zool.*, 1917, *b*, xxii, 471. Laurens, H., and Hooker, H. D., Jr., *J. Exp. Zool.*, 1920, *c*, xxx, 345.
9. Gross, A. O., *J. Exp. Zool.*, 1913, xiv, 467. McEwen, R. S., *J. Exp. Zool.*, 1918, xxv, 49.
10. Crozier, W. J., *J. Gen. Physiol.*, 1920–21, iii, 57.

GROWTH OF MAIZE SEEDLINGS AS AFFECTED BY GLUCOKININ AND INSULIN.

By W. H. EYSTER AND M. M. ELLIS.

(From the University of Missouri, Columbia.)

(Received for publication, April 19, 1924.)

As the nutrition and growth of young maize seedlings involve the transformation of a relatively large quantity of stored starch into glucose and related carbohydrates, and the utilization of this carbohydrate material—conditions at least analogous to the liberation and utilization of blood sugar in the animal body—maize seedlings were treated with glucokinin or with insulin to determine whether the utilization of carbohydrates could be influenced in plants as in animals. Qualitative experiments have been reported previously (Ellis and Eyster (1923)) showing that maize seedlings are affected in a number of ways when treated with glucokinin or insulin. In the present paper quantitative changes in growth of very young maize seedlings are considered. Although growth is not dependent upon the consumption of carbohydrates alone, growth has been used as an index of general metabolism, and, therefore, indirectly of the carbohydrate metabolism of the plant, as the energy of the seedling was expended very largely in growth during the period through which the carbohydrates of the endosperm were being consumed.

From preliminary experiments it was found that the seedlings of ordinary maize varied so greatly in the amount of growth as to be unsuitable for quantitative studies. In all of the experiments reported, therefore, only young seedlings of pedigreed strains of maize, most of which had been inbred for four or more generations were used. Seedlings of such strains were remarkably uniform, although those of one strain were often different from those of another inbred strain in both roots and tops. In each experiment seedlings from a single strain, in many cases from a single ear, were used so that all might be directly comparable.

The grains were surface sterilized with calcium hypochlorite (Wilson (1915)) and then germinated either in sand or on moist filter paper. In from 5 to 7 days, when the primary roots of the seedlings were from 2 to 4 inches in length, the seedlings were carefully removed and placed in large flat trays for inspection preliminary to the selection of the plants that were to be used in the experiment. Any plants that differed from the general average in size or other respects, were rejected and the remainder separated into as many lots as were required for the several series of the experiment. Each seedling was transferred to a 25 cc. test-tube which contained 20 cc. of distilled water, or distilled water and known quantities of either glucokinin or insulin. Each plant was supported at the top of the test-tube by a bit of cotton which held the endosperm above the fluid. The tubes containing the seedlings were held upright in a pasteboard box by being placed in equally spaced holes in the lid of the box. The roots were also protected from the light in this way. All plants were kept in a greenhouse under conditions favorable for growth. This method of germinating the grains and growing the plants gave satisfactory results as evidenced by the fact that very few seedlings became diseased and had to be eliminated from the experiments.

The experiments extended over a period of from 3 to 8 days during which time the amount of fluid was kept approximately constant by the addition, from time to time, of distilled water to replace the water used by the plant or lost by evaporation. At the end of the experiment the weights of the top, root, and in some cases of the endosperm, of each individual plant were taken. Just before weighing each seedling was carefully blotted between dry filter papers to remove any fluid on the outside of the plant.

Experiments with Untreated Plants.

In order to facilitate the entrance of the test solutions into the plants, the tip (5 to 10 mm.) of the primary root of each seedling was cut off with a sharp razor just before the plant was put into the test solution, thus making it possible for the solution to enter the vascular system directly. The control plants of each experiment were similarly prepared, but to determine the effect of this procedure

on untreated plants, 95 carefully selected seedlings were grown in distilled water alone and the root and top growth of those having cut primary roots compared with those having entire roots. There were no observable differences between the tops of the cut and uncut series, but the seedlings with cut roots put out more lateral roots than those with uncut roots. This difference in root growth was evidently the result of the removal of the meristematic tissue at the tip of the primary root.

TABLE I.

Effect of Cutting Primary Root Tip on Growth of Tops and Roots of Untreated Seedlings Grown in Distilled Water. Inbred Green Maize.

Series.	No. of seedlings.	Tip of primary root.	Length of test.	Average final weight.	
				Top.	Roots.
			days	*mg.*	*mg.*
Aa	8	Uncut.	4	510 ± 6.34	385 ± 6.14
Ab	9	Cut.	4	609 ± 7.92	445 ± 9.06
Ac	20	Uncut.	6	688 ± 8.10	436 ± 8.11
Ad	17	Cut.	6	635 ± 7.63	464 ± 9.02
Ba	20	Uncut.	5	631 ± 8.77	
Bb	21	Cut.	5	631 ± 7.96	

In this table and the following tables the first letter of the series designation refers to the strain and the second letter to the treatment. All plants belonging to series having the same initial letter are therefore directly comparable as regards pedigree.

The actual weights (Table I) show that the series with cut primary roots had made 17 per cent more top growth and 12 per cent more root growth than the uncut series at the end of the 4th day; that the top weights were the same for both series at the end of the 5th day; and that the cut series was 8 per cent behind the uncut series in top growth although 6 per cent ahead of the uncut series in root growth at the end of the 6th day. These results indicate that the removal of the tip of the primary root did not seriously injure the seedlings and in the following experiments the tip of the primary root of each plant was cut off at the time of treatment unless otherwise specified.

Experiments with Glucokinin.

The glucokinin was prepared by Collip's method (1923) from onion tops and from young maize plants. Both lots of glucokinin were tested to prove their potency in reducing blood sugar of animals, but no attempt was made to standardize the glucokinin in terms of rabbit-insulin units. The relative strengths of solutions used have been expressed in per cent of crude dry glucokinin dissolved in distilled water (Table II).

TABLE II.

Effect of Crude Glucokinin on Top Growth.

Length of test.	Untreated.			Treated with glucokinin.				(*b* − *a*)
	Series.	No. of seedlings.	Average final top weight (*a*).	Series.	No. of seedlings.	Glucokinin solution.	Average final top weight (*b*).	
days			*mg.*			*per cent*	*mg.*	*mg*
5	Bd	41	631 ± 7.96	Bc	9	0.5*	628 ± 8.21	−3 ± 11.44
5	Ca	17	354 ± 8.86	Cb	18	0.0005*	426 ± 8.93	72 ± 12.67
7	Ea	20	526 ± 8.33	Ec	20	0.02*	611 ± 8.72	85 ± 12.22
4	Ab	9	609 ± 7.92	Ae	4	0.5†	585 ± 7.99	−24 ± 11.43
6	Ad	17	635 ± 7.36	Af	10	0.5†	525 ± 8.11	−110 ± 11.34
3	Da	13	274 ± 9.02	Db	16	0.1†	286 ± 8.96	12 ± 12.94
3	Da	13	274 ± 9.02	Dc	15	0.05†	279 ± 9.66	5 ± 13.45
7	Ea	20	526 ± 8.33	Eb	10	0.001†	563 ± 7.89	37 ± 11.48

* Glucokinin prepared from onion tops.
† " " " corn plants.

As has been noted in previous preliminary experiments, in general the weaker solutions of glucokinin promoted growth of both tops and roots, and the stronger solutions depressed growth below the values found for normal untreated plants grown in distilled water. The tops of plants receiving amounts of glucokinin which promoted growth were sturdier and more vigorous than those of their untreated controls, while the tops of plants treated with stronger solutions of glucokinin were smaller, less green, and in many cases the leaves wilted at the tip and died. The contrast in root growth between controls and seedlings grown in strong glucokinin solutions was even more striking, the length and number of the roots being materially reduced, in fact very strong solutions practically stopped all root growth.

In view of the fact that glucokinin is similar to insulin it was thought that it might not be readily dialyzable as Dudley (1923) and others have shown to be the case with insulin, and, therefore, not easily absorbed by the plant. To test the ability of maize seedlings to absorb glucokinin, 194 plants representing four pedigreed strains were grown in two different solutions of this substance. Each comparable lot of seedlings was divided into two series, one composed of plants having the tip of the primary root removed as described above, and the other with roots intact. In every case the

TABLE III.

Comparison of Top Growth of Seedlings with Cut and Uncut Primary Roots Treated with Glucokinin.

Length of test.	Glucokinin used.	Primary root cut.			Primary root uncut.			(a − b)
		Series.	No. of seedlings.	Average final top weight (a).	Series.	No. of seedlings.	Average final top weight (b).	
days	*per cent*			*mg.*			*mg.*	*mg.*
5	*	Ga	18	586 ± 6.00				
5	0.005	Gb	20	673 ± 6.25	Gd	18	647 ± 5.34	26 ± 8.54
5	0.5	Gc	9	628 ± 6.43	Ge	11	551 ± 5.49	77 ± 8.71
5	0.005	Ha	20	475 ± 5.44	Hb	20	430 ± 6.27	45 ± 8.30
5	0.5	Hc	14	497 ± 6.04	Hd	10	399 ± 4.12	98 ± 7.31
7	0.005	Fa	10	568 ± 5.93	Fb	8	514 ± 6.80	54 ± 9.03
7	0.005	Ia	18	426 ± 6.34	Ib	18	335 ± 6.53	91 ± 9.10

* Controls, grown in distilled water.

plants with cut roots made greater growth in tops than the seedlings with uncut roots grown in the same strength solution of glucokinin (Table III).

Seedlings from Ear 1089-3 (Series Ga, Gb, Gc, Gd, Ge) furnish additional comparisons as 18 of them (Ga) with roots cut, were grown in distilled water alone. The average top weight of these 18 seedlings at the end of 5 days was 586 mg. If this value be compared with the average top weights of the cut but treated plants (Gb, Gc) from this ear, it may be seen that the average top weight of those seedlings

grown in a 0.005 per cent glucokinin solution exceeded this weight of Series Ga by 87 mg., and of those grown in 0.5 per cent solution, Gc, by 42 mg. The average top weight of Series Gd, plants with uncut roots grown in 0.005 per cent glucokinin also exceeded the average top weight of Series Ga by 61 mg., but the average top weight of the seedlings of Series Ge, those with uncut roots grown in 0.5 per cent glucokinin, was 35 mg. less than that of the plants with cut roots grown in distilled water, Series Ga. It has been pointed out in Table I that the top growth of seedlings comparable with those of Series G was the same for plants with cut and uncut roots grown in distilled water, at the end of the 5th day. Consequently the difference in top weight at the end of 5 days between the treated seedlings with uncut roots, Series Ge, and those with cut roots grown in distilled water, Ga, must have been due to the action of the glucokinin solution. These results indicate that the growth-promoting substance in the glucokinin solutions was not taken into the plants with uncut roots as rapidly as it was into those with cut roots, presumably by way of the vascular bundles, and also that the solutions of crude glucokinin contained other substances in addition to that promoting growth. The poorer top growth of the seedlings with uncut roots grown in 0.5 per cent glucokinin as compared either with the controls in distilled water or with those seedlings with cut roots grown in 0.5 per cent glucokinin was probably due to the more rapid entry by dialysis of some of the substances in the solution of crude glucokinin other than the growth-promoting substance, which substances, as was shown in another set of experiments, repressed growth. In the case of Series Gc, the plants with cut roots treated with 0.5 per cent glucokinin solution, all of the substances in the solution could enter the plant freely through the vascular bundles, and the action of the growth-promoting substance exceeded that of the other substances.

To verify the conclusion that the more rapid absorption of some substances from the crude glucokinin was responsible for the repressed growth of the seedlings with uncut roots grown in the stronger solutions of glucokinin, experiments were made with dialyzed crude glucokinin. Strong solutions of crude glucokinin were placed in celloidin cells and dialyzed against twice their volumes of distilled water for 18 hours. In the course of this dialysis the non-dialyzable residue

stained the celloidin a deep coffee-brown color, so that the solution of residue at the end of the 18 hours was clear and almost colorless, in contrast to the light brown color of the stronger solutions of undialyzed glucokinin. The dialysates were also clear and colorless. Preliminary experiments showed that both residue and dialysate affected the growth and metabolism of maize seedlings (Ellis and Eyster (1923)). Seedlings treated with glucokinin residue not only made better growth than the untreated controls but showed an increased chlorophyll production. On the other hand, seedlings treated with glucokinin dialysate in many cases exhibited a yellowing and wilting of the leaves, and from the tips of the leaves of the seedlings grown in strong solutions of onion glucokinin dialysate drops of sticky, brown fluid containing glucose were exuded.

In Table IV comparisons of root growth and top growth of seedlings grown in glucokinin residue and dialysate, respectively, are given. The plants treated with glucokinin residue always exceeded those grown in glucokinin dialysate solution of the same strength, as well as those grown in distilled water, in both root growth and top growth. Plants treated with glucokinin dialysate, although making less gain in growth than the glucokinin residue plants, frequently exceeded the growth of the plants grown in distilled water. In some cases growth of both tops and roots was repressed by the dialysate solutions.

That the increase in growth produced by the glucokinin residue solutions was due to the presence of purified glucokinin seems evident and was substantiated by all of the tests made either with purified glucokinin or with purified insulin. The less vigorous growth of plants treated with the glucokinin dialysate solutions may have been due to the action of some substance carried over from the raw plant material in the course of the preparation of the glucokinin, or to some substance added during this process. The method followed in the preparation of glucokinin involved the use of but one substance which is not a volatile solvent, namely ammonium sulfate. This salt being crystalline, if carried over in the manufacture of the crude glucokinin, would appear in the dialysate. Any other substance occuring in the dialysate would necessarily come from the plant tissue from which the glucokinin had been extracted.

TABLE IV.

Comparison of Effects of Glucokinin Residue and Glucokinin Dialysate on Root Growth and Top Growth.

Length of test.	Series.	No. of plants.	Treatment.	Average final weight.		Difference between final weight and that of untreated controls.	
				Top.	Roots.	Top.	Roots.
days				*mg.*	*mg.*	*mg.*	*mg.*
4	Ab	9	*	609 ± 7.92	445 ± 9.06		
4	Ag	15	0.5% dialysate.	635 ± 9.61	455 ± 8.26	26 ± 12.4	10 ± 12.2
4	Ai	16	0.5% residue.	751 ± 8.97	612 ± 9.44	142 ± 11.9	167 ± 13.1
6	Ad	17	*	635 ± 7.63	464 ± 9.02		
6	Ah	14	0.5% dialysate.	674 ± 8.75	375 ± 10.17	39 ± 11.6	−89 ± 13.6
6	Aj	15	0.5% residue.	776 ± 8.55	623 ± 9.63	141 ± 11.5	159 ± 13.2
5	Bb	21	*	631 ± 8.77			
5	Bd	16	0.5% dialysate.	708 ± 8.84		77 ± 12.5	
5	Be	20	0.005% residue.	720 ± 9.01		89 ± 12.6	
6	Ja	35	*	432 ± 9.23			
6	Jb	21	0.1% dialysate.	452 ± 9.66		20 ± 13.4	
6	Jc	23	0.1% residue.	510 ± 9.54		78 ± 13.2	
4	Ka	21	*	638 ± 10.60	470 ± 12.06		
4	Kb	15	0.05% Fract. "J."	687 ± 12.80	340 ± 11.14	49 ± 16.6	−130 ± 16.4
4	Kc†	18	0.05% Fract. "K."	514 ± 10.40	286 ± 18.14	−124 ± 14.8	−184 ± 21.5
4	Ke†	17	0.05% $(NH_4)_2SO_4$.	468 ± 12.60	252 ± 15.12	−170 ± 16.5	−218 ± 19.3
6	Kg	16	*	664 ± 9.16	495 ± 10.21		
6	Kd†	16	0.05% Fract. "K."	307 ± 15.86	282 ± 16.71	−357 ± 18.4	−213 ± 19.6
6	Kf†	15	0.05% $(NH_4)_2SO_4$.	316 ± 14.97	179 ± 17.78	−348 ± 17.6	−316 ± 20.5
4	La	14	*	625 ± 8.61	424 ± 9.02		
4	Lb†	17	0.01% $(NH_4)_2SO_4$.	458 ± 10.23	390 ± 11.71	−167 ± 13.4	−34 ± 14.8

* Controls, grown in distilled water.
† Plants wilted as result of treatment.

To determine the source of the active substances found in the glucokinin dialysate two separations were made. The crude glucokinin solution was treated with ten volumes of 95 per cent alcohol and a crystalline precipitate (Fraction "K") obtained. Glucokinin dialysate solution was also treated with nine volumes of 95 per cent alcohol and another crystalline precipitate recovered (Fraction "J"). Both precipitates when redissolved and recrystallized, showed the presence of ammonium sulfate. In Table IV the effect of these two fractions and of two solutions of C.P. ammonium sulfate on the growth of maize seedlings is shown. Both of the ammonium sulfate solutions and Fraction "K" depressed root growth and top growth. Solutions of Fraction "J" promoted the top growth but repressed root growth below that of the distilled water controls. Neither of these two fractions nor the ammonium sulfate solutions had any noticeable effect on the chlorophyll content of the leaves, nor did they cause the exudation of the glucose-containing fluid from the tips of the leaves as was noted in plants treated with dialysate solution of onion glucokinin. From these results it seems probable that at least three substances were included in the solutions of crude glucokinin: (1) ammonium sulfate, carried over in the manufacture of the glucokinin which repressed growth, (2) some active principle which stimulated growth, and (3) some substance or substances which disturbed the sugar metabolism as previously described. It is entirely possible that the growth-promoting substance in the dialysate may have been a small amount of the growth-promoting substance of the residue which had passed through the celloidin membrane.

Experiments with Insulin.

Crude insulin was prepared from beef pancreas and hog pancreas by the alcohol method without the use of acid. As in the case of the crude glucokinin used in these experiments, all preparations of insulin were tested on animals to prove their potency in lowering blood sugar, but the crude insulin was not standardized in terms of rabbit-insulin units. The relative strengths of the crude insulins are given in per cent of dry insulin in solution. For highly purified and standardized insulin we are indebted to Dr. G. H. A. Clowes of the Lilly Research Laboratories.

† Plants wilted as result of treatment.

The results obtained with insulin uniformly were consistent with those obtained with glucokinin, the weaker solutions promoting growth and the very strong solutions repressing it. When crude insulin, or even the highly purified insulin, was dialyzed the residue was more effective in promoting growth than the dialysate. The dialysate solutions may have been less effective in promoting growth than the residue solutions merely because the dialysates contained less insulin, which dialyzes with difficulty, or because of the combined action of such insulin as did pass through the celloidin membrane together with other dialyzable substances present in the insulin solution. As neither ammonium sulfate nor acid were used in the preparation of the insulin, the substances repressing growth of plants treated with strong solutions must have come from the animal tissues from which the insulin was prepared. In Table V are given the summarized results of experiments with insulin involving over 500 seedlings of seven inbred strains of maize, including normal greens, pale greens, and albinos. In these experiments the treated plants, without exception, showed greater top growth and root growth at the end of 4 to 7 day periods than their controls grown in distilled water.

As the results of all the experiments indicated that treated plants made better growth in both tops and roots, provided that the amount of insulin given was not too large, a series of seedlings was treated with highly purified insulin to determine whether the increase in growth was correlated with the utilization of stored food in the endosperm. 195 seedlings from the same ear of maize of a strain that had been inbred for four generations were selected with great care for uniform size and condition, and divided into six approximately equal lots. The total weight, and the weights of the top, root, and endosperm of each plant in Series Sa were taken at the beginning of the experiment. The total weight of each plant in each of the five remaining series, Sb to Sf, was also taken at this time. The seedlings were then treated as in the previous experiments and grown in distilled water, Series Sb, in two strengths of insulin, Sc and Sd, in residue solution, Se, and in dialysate solution, Sf. The strengths of the solutions used are given in terms of rabbit-insulin units, as only the highly purified and standarized insulin was used in this test.

TABLE V.

Effect of Insulin on Top Growth and Root Growth.

Length of test.	Series.	No. of plants.	Treatment.	Average final weight.		Difference between final weight and that of untreated controls.	
				Top.	Roots.	Top.	Roots.
days				*mg.*	*mg.*	*mg.*	*mg.*
7	Qc	37	0.01% crude hog insulin.	538 ± 6.82		45 ± 9.73	
7	Qa	22	*	493 ± 6.94			
7	Qb	34	0.1% crude hog insulin.	560 ± 7.41		67 ± 10.15	
6	Ad	17	*	635 ± 7.63	464 ± 9.02		
6	Ak	24	0.1% crude hog insulin.	691 ± 7.62	505 ± 9.41	56 ± 10.78	41 ± 13.04
4	Ab	9	*	609 ± 7.92	445 ± 9.06		
4	Al	17	0.2% crude hog insulin.	623 ± 7.83	501 ± 8.78	14 ± 11.14	56 ± 12.60
6	Ma	18	*	586 ± 8.62			
6	Mb	20	0.05% crude beef insulin.	659 ± 8.89		73 ± 12.38	
4	Na†	26	*	453 ± 9.14			
4	Nb†	36	0.05% crude beef insulin.	461 ± 8.47		8 ± 12.43	
5	Pa	35	*	567 ± 10.17			
5	Pb	36	0.05% crude beef insulin.	678 ± 9.63		111 ± 14.01	
5	Ra	11	*	480 ± 8.01	206 ± 10.11		
5	Rb	24	0.01% residue hog insulin.	600 ± 8.19	329 ± 7.64	120 ± 11.45	123 ± 12.67
5	Rc	23	0.01% dialysate hog insulin.	510 ± 7.43	226 ± 8.32	30 ± 10.92	20 ± 13.09

* Controls, grown in distilled water.

† Albino plants.

TABLE VI.

Weights in Mg. of Top, Root, and Endosperm of Plants in Series S at the End of the 6th Day of Treatment with Insulin.

Series.	No. of plants.	Treatment.	Top.	Roots.	Endosperm.	Comparison with Series Sb.		
						Top.	Roots.	Endosperm.
Sb	34	Distilled water.	454 ± 7.97	247 ± 8.45	171 ± 4.66			
Sc	34	Insulin 1 unit in 10 cc.	529 ± 10.06	327 ± 7.94	211 ± 7.44	175 ± 12.84	80 ± 11.57	40 ± 8.77
Sd	24	Insulin 1 unit in 26.5 cc.	433 ± 12.66	314 ± 11.13	223 ± 7.06	−21 ± 14.93	67 ± 13.92	72 ± 8.43
Se	21	Residue 1 unit in 20 cc.	570 ± 9.04	346 ± 12.53	219 ± 8.63	116 ± 12.04	99 ± 15.09	48 ± 9.79
Sf	40	Dialysate 1 unit in 20 cc.	438 ± 8.50	296 ± 8.95	210 ± 6.11	−16 ± 11.66	49 ± 12.28	39 ± 7.68

At the end of 6 days each plant was again weighed as a whole, cut apart, and the individual top, root, and endosperm weighed separately. In Table VI the mean weights of top, root, and endosperm for Series Sb to Sf are given. The difference between the mean weights of the top, root, and endopserm of the treated series, Sc to Sf, and the mean weight of the distilled water controls, Series Sb, are also tabulated. In Table VII the average gain in weight of top and root and the average loss in weight of the endosperm, in terms of the original weights of these parts are listed. The original weights of the top,

TABLE VII.

Loss of Weight of the Endosperm Compared with the Gain in Growth in Insulin-Treated Seedlings and Untreated Seedlings. Duration of Experiment, 6 Days. Per Cent Computed on Basis of Original Weight of the Parts. All Plants from Same Ear of Maize.

Series.	No. of plants.	Treatment.	Per cent gain in weight.		Per cent loss in weight.	Gross per cent gain in growth.	Net per cent gain in growth.	Gross gain in growth over controls, Series Sb.	Net gain in growth over controls, Series Sb.
			Tops.	Roots.	Endosperm.				
Sa	42	Weighed.							
Sb	34	Distilled water.	80	5	55	43	14		
Sc	34	Insulin 1 unit in 10 cc.	109	31	45	70	34	63	143
Sd	24	Insulin 1 unit in 26.5 cc.	82	50	31	66	47	53	236
Se	21	Residue 1 unit in 20 cc.	107	72	30	90	66	109	381
Sf	40	Dialysate 1 unit in 20 cc.	85	34	38	60	35	40	149

root, and endosperm for plants of Series Sb to Sf were computed from the original total weights of these plants by the use of the average per cent of the total weight represented by the weights of the top, root, and endosperm of the plants of Series Sa which were cut apart and weighed at the beginning of the experiment.

The tops of the plants of Series Sb, the controls, increased 80 per cent in weight and the roots 5 per cent during the 6 days of the experiment, and the endosperms of these plants lost 55 per cent of their weight. Compared with the growth made by these controls

in distilled water all of the plants of the treated series exceeded the controls in root growth, and those of Series Sc and Se, treated with the stronger solution of insulin and with insulin residue, respectively, also exceeded the controls in top growth. Series Sd and Sf, those plants grown in the weaker insulin solution and in the insulin dialysate solution, made slightly greater gains in top weight (82 and 85 per cent as compared with 80 per cent), than the controls, in terms of their original weights (Table VII). The average final top weights (Table VI) of Series Sd and Sf were, however, slightly less than the final top weight of Series Sb. As the probable errors of these differences show them to be too small to be significant the top growths of Series Sd and Sf were considered as approximately the same as that of Series Sb, the controls. The growth-promoting action of the insulin solutions was evident in all series of treated plants regardless of the strength of solution used, for even Series Sd and Sf made substantial gains in root growth. Plants treated with insulin residue made more than twice the gain in total growth and nearly four times the net gain in growth as compared with the untreated controls. Even those treated with dialysate solution, although merely equalling the controls in top weight made a gain of 40 per cent in total growth and 149 per cent in net growth over the controls. These values are all based on individual growth differences (see Table VI) which have been shown to be statistically significant as the average differences are all well above three times the probable error of the difference.

This action of insulin is more striking, however, if the total growth gain, that is the gain in growth of the roots and tops together, be compared with the loss of weight by the endosperm. Not only did the insulin-treated plants make greater gains in growth than did the untreated controls, but these gains were made with less loss of endosperm. As the only food available for these seedlings was that in the endosperm and such food as each plant was able to elaborate for itself, the insulin treated plants must have used the food from the endosperm more efficiently or have produced more food, photosynthetically, than the controls. Possibly both the utilization of food and the production of new food were affected by the insulin. The weight of the seedlings could have been increased only by growth,

by the absorption of water and by the insulin taken into the plant. The last possibility may be dismissed at once as the quantities of insulin used were very small, the strongest solution containing less than 20 mg. of dry substance per 20 cc. That the increase in weight was not due to the simple absorption of water may be checked by the linear measurements of the plants which showed proportional increases in length of roots, length and width of leaves, and diameter of stalk, correlated with the increases in weight, and also by the actual increase in the number of leaves and rootlets. All of these changes call for definite growth increases.

If all of the material represented by the loss in weight of the endosperm be considered as having been transformed without loss into tissue added during growth, and this weight be deducted from the weight of the roots and tops at the end of the experiment before the per cent of increase in growth be computed, the net gain in growth is obtained (Table VII). From these values it may be seen that even after deducting all of the weight lost by the endosperm from the gain in growth of the roots and tops together, the insulin-treated plants exceed the controls in net growth gains. As it is highly improbable that the material from the endosperm was transformed without loss into tissue, the actual differences in net growth grain were probably greater than those given in the table. This comparison of net growth gains merely states in another way that the endosperm was used more effectively by the treated plants, or that they were able to produce more food than the controls, or that there was some combination of both of these factors.

One series of albinos offers some evidence that the insulin-treated plants actually elaborated more food. Series Nb (Table V), albino plants treated with beef insulin, exceeded their untreated albino controls by but 8 mg. in top growth in a 4 day test. Series Pb, green segregates from the same ear, treated with the same amount of beef insulin exceeded their green controls, Series Pa, by 111 mg. in top growth in a 5 day test. Photosynthesis was not carried on by these albinos which were pure white and entirely free from either yellow or green pigment, and if insulin promotes the production of food either directly or indirectly through photosynthetic activity the albino plants could derive no benefit from that action of insulin.

The green plants may have made their greater growth either as the direct result of increased food production under the action of insulin or as a result of increased chlorophyll production (which has been shown to follow insulin treatment in some seedlings) which in turn might promote greater food production. Plants lacking both yellow and green pigments may have inherent physiological peculiarities which might account for the differences in the growth of albinos under insulin treatment as compared with the green segregates from the same ear, but in the absence of proof of such peculiarities the relatively slight effect of insulin on the top growth of these albinos seems suggestive.

Considering the separate series, the insulin residue solution was the most active in promoting growth. The residue of one rabbit unit of insulin in 20 cc. of distilled water after dialysis produced top growth approximately equalling that of plants grown in twice the strength of undialyzed insulin. The dialysate solution from the dialysis in which the residue solution was prepared produced no appreciable change in top growth although the root growth of plants grown in this solution was about the same as that of plants treated with a solution of undialyzed insulin containing one rabbit unit per 10 cc. These differences are of the same character as those already discussed under the dialysis of glucokinin. As the insulin used in this experiment was highly purified the increase in root growth of the plants grown in dialysate solution may have been the result of the presence of some of the growth-promoting substance which had passed through the celloidin into the dialysate.

As the purified insulin was preserved with a very small quantity of tricresol this substance was tested both on plants and animals in the dilutions in which it was present in the solutions used in Series Sc to Sf. No effect was observed. The pH of the distilled water and of the solutions in which all of the plants were grown was frequently taken. All solutions ranged from pH 6.8 to 7.3.

That the changes in growth produced by the various glucokinin and insulin solutions were not the result of differences in nutritive value of the solutions used as compared with distilled water is apparently shown by several facts. The actual amount of material available in the strongest solution was too little to supply more than

part of the inorganic salt requirement at most. The experiments with plants having cut and uncut primary roots showed the same strength of solution to have promoted growth in plants with cut roots and repressed growth in plants with uncut roots, as compared with their distilled water controls (see Series G). Had the gain in growth been the result of inorganic salt supply the plants with uncut roots grown in glucokinin solutions should have made better growth than their distilled water controls. In several of the experiments with both glucokinin and insulin the treated plants made relatively better root growth than top growth suggesting that the growth gain was not made because of the presence of some iron salt which the distilled water did not contain. The experiments with albino plants in particular and all experiments in which the loss of endosperm was studied point to changes in metabolic activity rather than differences in external food supply. It seems probable, therefore, that the action of glucokinin and insulin in promoting growth of maize seedlings is of a specific character rather than one caused by differences in external, available food.

SUMMARY.

1. Solutions of glucokinin and insulin, particularly those from which the easily dialyzable substances had been removed, increased the growth of roots and tops of young maize seedlings, as shown by comparisons with untreated seedlings grown in distilled water.

2. Strong solutions of crude glucokinin or of crude insulin repressed growth.

3. Seedlings from which the tips of the primary roots had been removed just before placing the plants in the test solutions made greater gains in both top growth and root growth than seedlings with uncut roots treated with solutions of the same strength. Control experiments showed that this difference in growth was not the result of cutting the roots, and that crude glucokinin and crude insulin contained several substances some of which were more readily absorbed by the plant than others.

4. Purification of crude glucokinin and crude insulin by dialysis showed that the residue of relatively non-dialyzable substance was the growth-promoting fraction.

5. The dialysate of crude glucokinin contained at least three types of material, one of which repressed growth.

6. Ammonium sulfate, one of the possible impurities of glucokinin, repressed the growth of seedlings but did not produce the other changes in metabolism shown by seedlings treated with dialysate of onion glucokinin.

7. The endosperm of plants treated with growth-promoting solutions of purified insulin did not lose weight as rapidly as the endosperms of untreated plants, indicating that the treated plants made their greater gains in growth by more efficient utilization of the endosperm, or as a result of greater photosynthetic activity, or by a combination of these.

8. Experiments with albino seedlings suggested that the greater gain in weight made by plants treated with insulin was the result in part of increased photosynthetic activity.

BIBLIOGRAPHY.

Collip, J. B., *J. Biol. Chem.*, 1923, lvii, 65.
Dudley, H. W., *Biochem., J.*, 1923, xvii, 376.
Ellis, M. M., and Eyster, W. H., *Science*, 1923, lviii, 541.
Wilson, J. K., *Am. J. Bot.*, 1915, ii, 420.

THE EFFECT OF ULTRA-VIOLET, X-RAY, AND RADIUM RADIATION ON THE TOXICITY OF NORMAL BLOOD.*

By DAVID I. MACHT and EBEN C. HILL.

(From the Laboratories of Pharmacology and Anatomy, Johns Hopkins University, Baltimore.)

(Received for publication, May 2, 1924.)

INTRODUCTION.

One of the authors in cooperation with various assistants has during the past few years been developing the new field of phytopharmacology, that is studying the effects of various drugs and poisons on plant tissues. This study has already yielded interesting results inasmuch as a comparison of the reactions of animal tissues to various drugs with the effect of the same substances on plant tissues gave very divergent findings. Thus, it was found that various substances which were very little, or not at all, toxic for zoopharmacological preparations were very toxic indeed for phytopharmacological preparations. For instance, in a study of cocaine and its decomposition products it was found that while the cocaine molecule was very toxic for animal protoplasm it was comparatively much less toxic for plant protoplasm (1). On the other hand, sodium benzoate which in small doses has no effect on animal tissues and is tolerated by animals even in very large doses, was very poisonous for certain seedlings which were studied. Again the phytopharmacological method was found to be especially adapted for the study of certain toxins such as menstrual toxin occurring in human blood (2). As a result of such observations it occurred to the authors that various changes in normal blood not ordinarily detectable by zoophysiological or zoopharmacological methods might be more easily detected by the use of phytophysiological or phytopharmaco-

* This investigation has been endowed in part by a grant from The Rockefeller Institute for Medical Research.

logical methods. In the present investigation the toxicity of normal blood for plant protoplasm was studied under normal conditions on the one hand and after radiation with rays of different wave lengths on the other hand.

Method.

The method employed was the same as that used by Macht and Livingston, and Macht and Lubin in other investigations. The effect of solutions of blood was studied on the growth of living seedlings of *Lupinus albus*. The procedure is described in detail in the above mentioned papers. Briefly, it consisted in growing a series of seedlings in a perfect nutrient medium such as Shive solution containing various salts necessary for the growth of the plants and comparing the growth of these plants with another series of seedlings suspended in a similar nutrient solution to which a small quantity of an unknown toxin or drug had been added. The increment in the growth of the well demarcated single straight rootlets can be easily measured and compared in such experiments. In the present investigation most of the experiments were made with defibrinated pig blood, a few experiments were also made with defibrinated ox blood, and still others were performed with human blood kept from coagulating by the addition of small quantities of heparin. Whenever an anticoagulant was used in studying blood, control experiments were made with normal nutrient solution containing the same concentration of the anticoagulant.

Experimental Results.

Effect of Ultra-Violet Radiation.—A series of experiments was made to determine the effect of ultra-violet radiation on the pharmacodynamic reactions of normal blood. This was found to be necessary in connection with a series of other investigations by one of the authors on the effects of carbon monoxide poisoning described elsewhere (3). The radiations were made with a Hanovia Alpine Sun mercury vapor quartz lamp by exposing 10 cc. of blood in an open glass dish, 5 cm. in diameter, at a distance of from 50 to 100 cm. Such radiations were given over a period ranging from 5 to 30 minutes. The toxicity of the blood after radiation was compared with the

toxicity of a sample of the same blood which had not been radiated by observing the effect of a 1 per cent solution in equal parts of distilled water and Shive solution on the growth of *Lupinus albus* seedlings.

The results obtained were quite definite and are exhibited in Table I. This table gives the *average* increment in the length of the roots of twenty seedlings for each experiment, at temperature of 22°C. at the end of 18 hours. It was found that ultra-violet radiation had no effect on the toxicity of blood for plant protoplasm and in some cases it actually rendered the blood less toxic.

Effect of X-Ray Radiation.—Many unrecorded experiments were necessary in considering the varied factors entering into the radiation of blood and blood serum. Results as to the length of exposure, the type of rays used, and comparison of Coolidge and gas tube rays had to be studied.

Tests of long exposure and low currents, shortened time with increased radiation, and almost instantaneous exposure (0.5 second) with a spark-gap at 10 inches and using 140 milliamperes were of interest and would justify many more experiments. As it is, however, this brief report does not justify more than very curtailed details. Soft rays did not penetrate to the depth of the fluid. Medium rays took about 2 minutes to affect the blood or serum so that the changes were demonstrable on the plant tissues.

The determination of any differences between rays from a gas or Coolidge tube demanded that both tubes be given great amounts of electric current. As a basis the spark-gap, the meter readings, and the results shown upon the same plate by radiographing the humerus were used. After setting the Coolidge and gas tubes at the points to give out the same milliamperage and spark control the gas tube was adjusted by a slight hardening or softening. A well seasoned nitrogen tube was used and little change had to be made. Then the Coolidge tube was adjusted, one half of an 8 × 10 plate was covered with lead, and the head and shaft of the humerus were radiographed on the unexposed portion by a gas tube. Immediately after this the lead plate was placed over the exposed portion of the plate and the Coolidge tube was used to radiograph the same bone. The time of exposure was the same. Development of the plate

TABLE I.

Growth of Lupinus Roots.

Experiment No.	Kind of blood.	Kind of radiation.	Normal growth.			Growth after radiation.		
			Whole blood.	Cells.	Plasma.	Whole blood.	Cells.	Plasma.
			mm.	mm.	mm.	mm.	mm.	mm.
1	Pig.	Ultra-violet; quartz lamp.	7.6			7.6		
2	"	" " " "	10.3			10.3		
3	"	" " " "	18.7			18.5		
4	"	" " " "	12.9			13.0		
5	"	" " " "	9.9			10.0		
6	"	" " " "	8.5			8.5		
7	"	" " " "			9.0			8.9
8	"	" " " "	13.2			13.2		
9	"	" " " "	9.3			10.1		
10	"	" " " "		8.1	8.8		8.5	9.2
11	"	" " " "	10.3			10.3		
12	"	Radium emanations.	9.8			7.6		
13	"	" "	19.5			19.0		
14	"	" "	12.3			10.0		
15	"	" "		16.8	21.0		15.4	11.6
16	"	X-ray, gas tube.	10.4			8.5		
17	"	" " "	14.4			14.0		
18	"	" " "	20.6			18.8		
19	Ox.	" " "	15.6			15.2		
20	"	" " "	12.9			12.0		
21	Pig.	" " "	13.6			12.2		
22	"	" " "			12.0			10.6
23	Human.	" " "	18.0		20.4	17.4		18.8
24	Ox.	" " "	20.0		17.0	17.0		16.0
25	Pig.	" " "		17.6	19.1		15.4	16.6
26	"	" Coolidge "	16.0			12.4		
27	"	" " "	14.7			12.0		
28	"	" gas "	10.9			10.6		
29	"	" Coolidge "	10.9			9.1		
30	"	" gas "	12.3			10.8		
31	"	" Coolidge "	12.3			10.0		
32	"	" gas "		12.0	11.2		11.7	10.7
33	"	" Coolidge "		12.0	11.2		11.1	10.0
34	"	" gas "		5.0	5.6		4.7	3.6
35	"	" Coolidge "		5.0	5.6		4.2	3.2

indicated any differences in penetration and gave a fairly accurate idea of the quality of the rays. A decision of 2 minutes for radiation of the blood was partially empirical and depended to a certain extent on injury to the tubes by strain or over heating. By dividing this time of 2 minutes into three periods both tubes were uninjured in any way and being set aside for these experiments alone, needed little adjustment. Only once was it necessary to readjust a tube during radiation and in this instance a lead plate protected the blood.

The machine used was a powerful Wappler equipped for both gas and Coolidge tubes. The following standard was used.

Gas tube, seasoned, nitrogen.	Coolidge tube.
4 inch spark-gap.	4½ inch spark-gap.
55 kilowatts.	57 kilowatts.
30 milliamperes to tube.	30 milliamperes to tube.
54 cm. from center of focal point to surface of fluid. Focal point 2 mm.	4 volts used for heating filaments.
2 minutes time in periods of 40 seconds each.	2 minutes time in periods of 40 seconds each.

Controls in X-Ray Radiation.—In the experimental stage it appeared that pouring blood from a small Erlenmeyer flask to the dishes, radiating and then pouring back the fluid into the flask had some slight effect on the results obtained. So two or three dishes equal in size were filled to an equal depth. These were continually covered except during exposure. Any possible effect from secondary rays to the control was prevented by lead protection. The same precautions were maintained when one specimen of blood was to receive a Coolidge tube radiation and another specimen a gas tube radiation. Specimens radiated were immediately covered and placed with the control in a safe place. During the radiations the electric meters were carefully watched and later the tubes were tested for any marked changes if a target focal point had heated.

Effect of Radium.—A few experiments were performed by exposing samples of blood to radium emanations. Emanations of radium were obtained through the courtesy of Dr. Howard A. Kelly and Dr. Curtis F. Burnam. These emanations were contained in small glass pearls which were dropped into tubes containing 10 cc. of blood

and broken, after which the tubes were tightly stoppered with rubber stoppers. After exposure to the emanations for a period of 1 hour, the toxicity of the blood samples was compared with normal blood. Doses of from 5 to 8 millicuries were used.

Only a few experiments with radium were made but these definitely indicated that the effect produced on blood was very much the same as that obtained with Roentgen rays. The blood was rendered more toxic and the toxicity was chiefly confined to the plasma. Another striking feature in all the radium experiments was a distinct hemolysis produced in the blood.

SUMMARY.

While the number of experiments performed is perhaps somewhat limited the results obtained were quite definite and warrant the following conclusions. The toxicity of normal blood for living plant protoplasm as studied on the growth of *Lupinus albus* seedlings is definitely influenced by various radiations. Ultra-violet rays produce no effect on normal blood or may even render it slightly less toxic. Roentgen rays render normal blood more toxic. The toxicity is greater in the case of the blood plasma as compared with the blood cells and a more toxic effect is produced with the Coolidge tube as compared with the gas tube. Radium emanations in the few experiments performed produced changes very much the same as those given by the x-rays.

BIBLIOGRAPHY.

1. Macht, D. I., and Livingston, M. B., *J. Gen. Physiol.*, 1921–22, iv, 573.
2. Macht, D. I., and Lubin, D. S., *J. Pharmacol. and Exp. Therap.*, 1924, xxii, 413.
3. Macht, D. I., Blackman, S. S., Jr., and Kelly, E. B., *Proc. Soc. Exp. Biol. and Med.*, 1923–24, xxi, 289.

THE INFLUENCE OF ELECTROLYTES ON POLLEN TUBE DEVELOPMENT.*

By R. A. BRINK.

(From the Department of Genetics, University of Wisconsin, Madison.)

(Received for publication, May 3, 1924.)

Cultures on artificial media have shown that pollen is highly susceptive to the injurious action of various neutral salts. Lidforss[1] drew attention to this and his observations have been confirmed and extended by Tokugawa.[2] The extreme sensitiveness of pollen in this regard led the writer to undertake a fuller study of the effect of electrolytes on germination and growth. The present paper embodies in summary form the results of experiments with reference to (1) the toxicities of different single salts, (2) the antagonism shown by certain combinations of these salts, and (3) the influence of hydrogen ions, the measurement of which was made possible by the knowledge gained of the foregoing relations.

Sucrose dissolved in carefully distilled water formed the basis of the culture media. The highest grades of chemicals were employed and while sterile conditions were not observed, precautions were taken to avoid disturbances on this account. The pollen was cultivated in hanging drops in closed cells at 25°C.

Experiments with sweet pea pollen showed that NaCl, KCl, and LiCl do not differ greatly in their toxicities. In concentrations ranging from 0.01 to 0.0066 M they inhibit germination entirely; at 0.001 M NaCl and KCl decrease growth by more than one-half. Even at 0.0002 M, NaCl reduces the length of sweet pea pollen tubes 15 per cent. Similar results were obtained with *Nicotiana* pollen, although

*Papers from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin, No. 44. Published with the approval of the Director of the Station.

[1] Lidforss, B., *Jahrb. wissensch. Bot.*, 1896, xxix, 1.

[2] Tokugawa, Y., *J. College Sc., Tokyo Imp. Univ.*, 1914, xxxv, Article 8.

KCl appeared to be somewhat less injurious. Tests with different sodium salts indicate that it is the cation which exerts the toxic action.

$BaCl_2$ and $MgCl_2$ proved to be roughly fifteen times as injurious to sweet pea pollen as NaCl, the relative toxicities of these substances being determined by the amounts necessary to just prevent germination. That the nature of the cation as well as its valency must be considered, however, is shown very clearly by the results with $CaCl_2$. After having tried several single salts with the invariable result that they were highly injurious it was very interesting to find that $CaCl_2$ in corresponding concentrations markedly promotes the development of sweet pea pollen tubes. Cultures containing this salt in a concentration of 0.01 M gave tubes nearly 50 per cent longer than the control in salt-free medium. Comparative tests with $CaCl_2$ $Ca(NO_3)_2$, and $CaSO_4$ showed that in general these salts were very similar in their growth-promoting effects, although at 0.002 M, $CaSO_4$ gave consistently higher values than the other two salts, a fact of possible significance.

With *Nicotiana* pollen, $CaCl_2$ proved relatively toxic but high per cent of germination and strong growth were repeatedly obtained in cultures containing $MgCl_2$ in a concentration of 0.002 M. The average length of the tubes was somewhat less at 0.01 M. The similarity of the effect of $MgCl_2$ on *Nicotiana* and $CaCl_2$ on sweet pea suggests that their action in the two cases may be analogous.

Well marked cases of antagonism were found between calcium and sodium and between calcium and potassium. The addition of 0.04 M $CaCl_2$ to sucrose cultures gives no greater growth than sucrose alone while the addition of NaCl in a concentration of 0.01 M entirely prevents germination. In cultures containing 0.01 M NaCl + 0.04 M $CaCl_2$, however, the pollen tubes of sweet pea averaged over 30 per cent longer than those in the pure sucrose medium. The injurious effect of 0.01 M NaCl is also overcome when the amount of $CaCl_2$ is reduced to 0.01 M. The average length of the tubes with this combination of salts was 192 per cent greater than in the accompanying salt-free sucrose cultures but 20 per cent less than when 0.01 M $CaCl_2$ alone was added. Comparable results were obtained when KCl replaced NaCl.

By taking advantage of the protective effect manifested by calcium in salt mixtures, media may be made in which the effect of varying hydrogen ion concentration can be measured. The most satisfactory

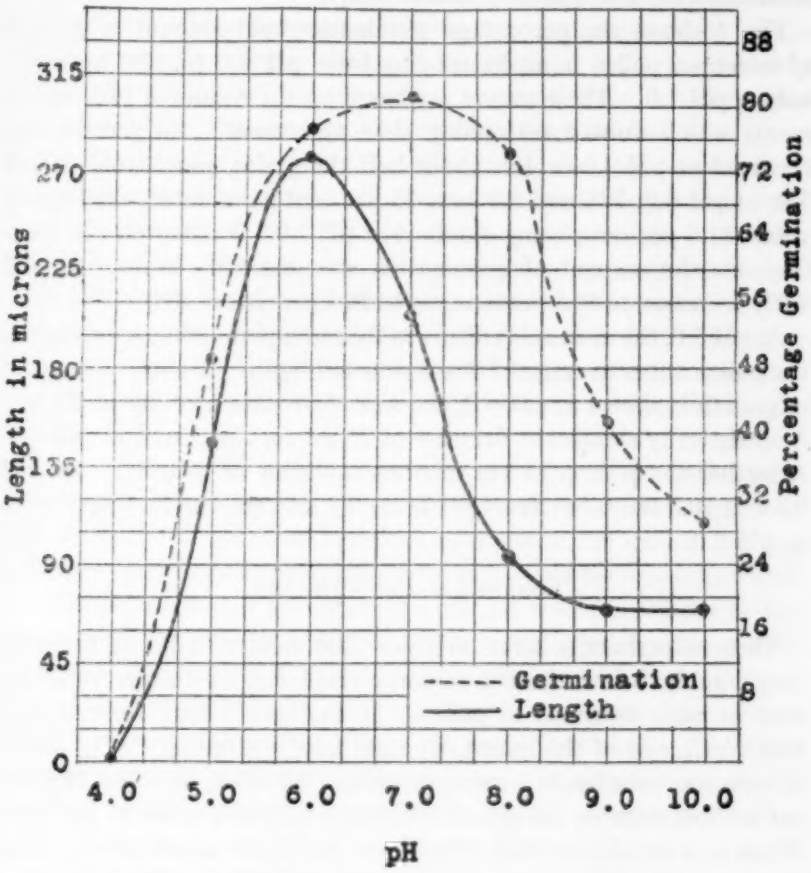


FIG. 1. Percentage germination and amount of pollen tube growth of sweet pea in relation to hydrogen ion concentration. The media were buffered with 3 per cent gelatin. Growth period about 6 hours. The growing pollen tube is appreciably more sensitive to pH than is the pollen grain.

results were obtained with cultures containing 3 per cent gelatin, the buffer effect of which was utilized in adjusting the pH with HCl and KOH. A highly purified gelatin (0.045 per cent ash) procured

from the Research Laboratory of the Eastman Kodak Company was employed. The addition of 0.01 M $CaCl_2$ served to inhibit the toxic effect of the K ions in the media. The pH values of the culture solutions were determined colorimetrically.

Fig. 1 shows the percentage germination and amount of growth of sweet pea pollen in media ranging from pH 4.0 to 10.0 by intervals of pH 1.0. These curves are based on the results of two experiments which showed reasonably close agreement. No germination occurred at pH 3.0 or 4.0; about half the grains germinated at pH 5.0; at pH 6.0, 7.0, and 8.0 over 75 per cent germinated, the highest value, 81.0 per cent, being obtained at pH 7.0. Farther on the alkaline side the amount of germination was distinctly lower. At pH 9.0 it decreased to 40.8 per cent and to 28.3 per cent at 10.0.

At pH 5.0, the most acid culture in the series permitting germination, the pollen tubes averaged 148 microns in length. A marked increase in growth is shown at pH 6.0, the most favorable medium in the set. A statistically significant decrease of 73 microns occurred at pH 7.0. A further sharp drop to 116 microns is shown at pH 8.0. At pH 9.0 and 10.0 the tubes averaged 21 $\pm$ 2.7 microns less in length than at pH 8.0.

DISCUSSION AND SUMMARY.

These experiments serve to show that neutral salts in amounts considerably below those commonly employed in culture solutions may be very injurious to pollen. It has been found, for example, that NaCl, one of the least toxic salts tried, excepting $CaCl_2$, added to a sucrose solution in a concentration of 0.0002 M, or about 11 parts per million, reduces the growth of sweet pea pollen tubes 15 per cent. When it is considered that $MgCl_2$ and $BaCl_2$ are about fifteen times as toxic as NaCl it becomes evident that the susceptibility of pollen tubes to injury by these substances amounts virtually to hypersensitiveness.

On the other hand calcium salts in concentrations ranging from 0.02 to 0.002 M markedly enhance the growth of sweet pea pollen tubes. $MgCl_2$ has a similar action in the case of *Nicotiana*. Calcium, moreover, exerts a strong protective action in the presence of the injurious monovalent cations Na and K.

So far as can be determined by microchemical means these salts do not alter the wall of the pollen tube; presumably, their effect is on the protoplast itself. In the light of recent experimentation (Osterhout[3]) with other forms better adapted to precise investigation of these phenomena it seems probable that the explanation of the facts presented here lies in changes brought about in the permeability of the cells. Since several gaps exist in our evidence, however, conclusions drawn at this time must necessarily be provisional.

The highly injurious action manifested by the cations of several of the salts used indicates that they penetrate the protoplast very rapidly. Possibly in pure sucrose cultures, exosmosis is a limiting factor in pollen tube growth. The addition of salts of calcium or magnesium may favor development by retarding or preventing this outward diffusion. The protective effect of calcium in the presence of the toxic cations K and Na is best interpreted on the assumption that the entry of these latter into the protoplast is retarded by the calcium.

The mode by which hydrogen ion concentration affects pollen tube growth is largely a matter of speculation. It has previously been been shown by Brink[4] that the time relations of the growth process simulate those of an autocatalytic reaction. It has been demonstrated also that elongation of the tubes in artificial media is related to the digestion of the reserve food materials contributed by the pollen grain. In the case of the sweet pea these stored substances are largely fats and their hydrolysis may constitute the most important chemical reaction in growth. If, as seems not improbable, the other reactions involved wait upon this one, it is the "master reaction" according to Robertson's[5] hypothesis. If this conception really applies to the case in hand as outlined, the effect of the concentration of hydrogen ions on growth may be a direct one. It is known that the action of the fat-splitting enzyme lipase is favored by a certain

[3] Osterhout, W. J. V., Injury, recovery, and death, in relation to conductivity and permeability, Monographs on experimental biology, Philadelphia and London, 1922, 259.

[4] Brink, R. A., *Am. J. Bot.*, 1924, xi, (in press).

[5] Robertson, T. B., The chemical basis of growth and senescence, Monographs on experimental biology, Philadelphia and London, 1923, p. viii, 389.

amount of free acid. The maximum rate of germination of the pollen and the greatest amount of growth of the pollen tubes occur at pH 6.0. This may be due in large part to the immediate effect of this concentration of hydrogen ions upon the digestion of the reserve food.

THE EFFECT OF POTASSIUM ON THE ACID METABOLISM OF SURVIVING SKELETAL AND CARDIAC MUSCLES OF THE FROG.

By FRED R. GRIFFITH, Jr.

(From the Laboratories of Physiology of the University of Buffalo, Buffalo, and Harvard Medical School, Boston.)

(Received for publication, May 5, 1924.)

Studies have been made of the effect of potassium on the oxygen consumption of developing eggs (1) and the carbon dioxide production of bacteria (2), but, apparently, its pronounced effect on the contractile process in muscle (3) has not been correlated with changes of metabolism of any kind.

This paper describes the effect of potassium on the acid production of surviving skeletal and cardiac muscles of the frog.

I.

Skeletal Muscle.

A. Total Acid Production.—In these experiments, sartorius muscles were used and their rate of acid production was measured by the indicator method of Haas (4).

Immediately after pithing a frog, its sartorius muscles were dissected out and a ligature tied around the tendon of insertion of each. This was done in order to have something by which the muscles could thereafter be handled without danger of stimulating them to contract. If a muscle was not to be used immediately it was left in a bath of several cc. of Ringer solution in a large watch-glass until needed.

In all experiments the "normal" rate of acid production was determined for each muscle in Ringer solution before subjecting it to the action of potassium. The Ringer solution used in this work had the following composition: sodium chloride, 0.763 per cent; potassium chloride, 0.014 per cent; calcium chloride, 0.012 per cent. The potassium effect was obtained with an isotonic, 1.08 per cent solution of potassium chloride. The pH values of the Ringer and potassium chloride solutions, as thus made up in distilled water, were 6.8 and 6.6, respectively, using brom-thymol blue as indicator.

In preparation for an experiment, a sample of each of these solutions was made alkaline with sodium bicarbonate. For this purpose, an approximately 0.5 M sodium bicarbonate solution was used in the proportion of 3 drops for each 100 cc. of Ringer or potassium chloride. Phenolsulfophthalein (5 drops of a 0.02 per cent aqueous solution per 10 cc.) was also added to each sample. The Ringer and potassium chloride solutions were then brought to the *same* hydrogen ion concentration (always pH 7.5±) by titration with 0.002 N hydrochloric acid. The samples, as thus prepared, were then allowed to stand in loosely stoppered Pyrex flasks for at least a day so as to permit them to come into equilibrium with the carbon dioxide tension of the laboratory air. Just before an experiment, any inequality of hydrogen ion concentration (as judged by the tint of the indicator in the solutions) was further equalized by titrating again with 0.002 N hydrochloric acid. The identical pH values and buffer capacities of the solutions thus prepared is evident from the fact that they maintained identical tints when exposed to laboratory air for several days; or when equilibrated with alveolar air (using methyl red as indicator); or when treated with equal amounts of 0.002 N hydrochloric acid. This last test was made as follows: 2.5 cc. of each solution were placed in small test-tubes, of the kind used in these experiments, and to each was added one drop of the acid; the test-tubes were then stoppered with paraffined corks and shaken. The same change of pH in the solutions of both tubes showed that they were identical as regarded their original hydrogen ion concentrations and buffer values.

The rate of acid production of the muscles and the effect of potassium on it were then determined as follows: 2.5 cc. of the adjusted Ringer solution were measured by a pipette into a small, hard glass test-tube of 3 cc. capacity; a muscle was lifted by its ligature from the bath of Ringer in which it was being kept, dried as much as possible of all adhering moisture by contact with filter paper, and dropped in the 2.5 cc. of Ringer solution in the test-tube; the tube was quickly stoppered with a paraffined cork and the time required to change the pH of the solution between standard[1] end-points of pH 7.381 and 7.168 determined with a stop-watch. During the determination the tube was continually tipped from end to end, holding it always by the stopper so as not to affect the temperature of its contents. At the end of a determination the contents of the tube, including the muscle, were poured into a watch-glass; the muscle was quickly picked up with forceps (by means of its tendon ligature), dried as before, and immediately placed in another 2.5 cc. of the Ringer solution and another determination made. This was repeated until the rate of acid production in Ringer solution was established. The muscle was then placed, in the same way and without any delay, in 2.5 cc. samples of the potassium chloride solution (previously adjusted as to pH and buffer values as described above) and determinations of the rate of acid production made under this condition. The transference of the muscle from one tube to another

[1] Phosphate standards prepared as described by Clark (Clark, W. M., The determination of hydrogen ions, Baltimore, 1920, 81) were used in this work.

required only about 30 seconds so the measurements of the rate of acid production made in this way give a fairly continuous picture of the variations in metabolic rate.

Twenty-six experiments, of which the two shown in Fig. 1 are typical, were all concordant in showing that the acid production of surviving sartorius muscles is increased when the muscles are transferred from Ringer to isotonic potassium chloride solution. The average, maximum increase for the entire series was about 200 per cent. This figure should be accepted, however, merely as indicating the qualitative nature of the effect because certain precautions that would have been necessary in order to make the experiments absolutely comparable quantitatively were not taken. For example, they were carried out at various room temperatures, which, though sufficiently constant during any one experiment, varied widely (14°–27°C.) on different days; also, in all but two of the experiments (those given in Fig. 1), no account was taken of the weight of the muscles so that the results could not be reduced to the common denominator of acid production per gm. of tissue; and finally, the potassium effect was tested at various times—15 minutes to 3½ hours—during survival. Yet the results are valuable just because they show that even under such diverse conditions potassium invariably increases the rate of survival acid production.

In all cases this increase attains its maximum promptly and is followed by a return to "normal." If the last determination in Ringer solution is taken as the "normal" rate it will be seen from Fig. 1 that the increased rate persists for 35 to 40 minutes. This, however, is probably too short a time; for it is not improbable that the course of total acid production (which may consist chiefly of carbon dioxide) follows approximately the course of survival carbon dioxide production; and this latter we know from the work of Fletcher (6) (see also Fig. 2) falls off rapidly during the first 2 hours of survival and then more slowly until death. The first part of the curve of Fig. 1 does indicate such a similarity between total acid production and carbon dioxide production during the 1st hour of survival and it is not unreasonable to suppose that the relationship would hold for the remainder of the survival period. In that case the curve representing the "normal" course of survival acid production would

intersect the curve of Fig. 1 sometime between 60 and 120 minutes, *i.e.* the increased rate of acid production caused by potassium persists for such a time.

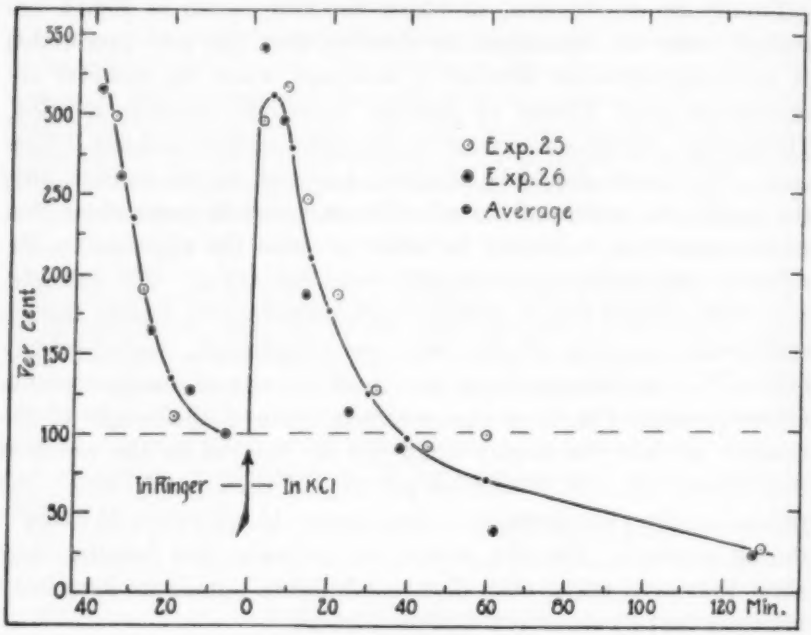


FIG. 1. The curve represents the average of two experiments in which sartorius muscles of the frog were treated with 1.08 per cent KCl near the end of the 1st hour of survival. Abscissæ, time in minutes; ordinates, rate of acid production expressed as per cent of the last determination in Ringer (100 per cent). Temperature throughout both experiments 19°–20°C. The complete data in regard to each experiment follow: in each case the figure in parenthesis is the time in seconds required for *1 gm.* of muscle to produce an increase in acidity from pH 7.381 to 7.168; the preceding figure is the number of minutes before or after immersion of the muscles in KCl (*i.e.* the abscissæ); the figure following the parenthesis is the rate of acid production calculated as a percentage of the final rate in Ringer solution (*i.e.* the ordinates).

Experiment 25.—Weight of muscle, 0.103 gm.; 34 (21.3) 298; 27 (33.2) 192; 19 (56.7) 111; 6 (63.7) 100; KCl; 3 (21.5) 296; 9 (20) 318; 14 (25.8) 246; 22 (34) 187; 32 (50) 127; 45 (69.2) 92; 60 (65.7) 98; 130 (123.6) 51; 310 (329.6) 19.

Experiment 26.—Weight of muscle, 0.083 gm.; 38 (14.9) 315; 33 (18) 216; 25 (28.15) 165; 15 (36.8) 127; 6 (46.8) 100; KCl; 3 (13.7) 342; 8 (15.8) 296; 14 (25.1) 187; 25 (38) 123; 38 (52.2) 90; 62 (123.8) 38; 128 (201.5) 23.

These results in regard to the effect of potassium on total acid production receive confirmation and are to some degree explained by the experiments that follow.

B. Carbon Dioxide Production.—The following experiments afford evidence that the increased acid production, recorded in the previous section, may consist entirely or at least in large part of carbon dioxide. These experiments were performed with a modified Osterhout apparatus which has previously been described (5) and by which carbon dioxide can be distinguished from other acids being produced at the same time. The technique was identical with that used in the work just referred to and therefore need be only briefly recapitulated here.

After pithing or decapitating a frog it was skinned and both hind legs retained after being cut off from the trunk. The leg muscles were cut loose at their insertions and separated from each other so as to permit as free access of the solution to all of them as possible. They were then immersed in 25 cc. of Ringer solution (adjusted for pH and buffer effect as described in the previous section) and the rate of carbon dioxide production measured, *i.e.* the time required to produce a change in acidity of the indicator solution (phenolsulfophthalein) from pH 7.381 to 7.168 was determined until the "normal" rate was established. The Ringer solution was then drained off and the muscles were treated with 25 cc. of 1.08 per cent potassium chloride solution, which, as described in the previous section, had been adjusted to the same hydrogen ion concentration and buffer capacity as the Ringer solution for which it was being substituted.

Ten experiments of this kind were performed at various room temperatures and at different times during survival, and all agreed in demonstrating an increased carbon dioxide production as a result of the potassium treatment. The data of four experiments are expressed graphically in Fig. 2.

The rate of carbon dioxide production by surviving frog muscles is known (6) to fall off rapidly during the first 2 hours to a fairly steady rate which is maintained with only a very gradual decrease thereafter. In all but three of the experiments of this series the potassium effect was tested at different times during this period of fairly uniform carbon dioxide production. Fig. 2, Curve II represents the only experiment of this type in which the normal course of carbon dioxide production was followed carefully from the beginning of survival; and it shows the effect of treating the muscles with potassium during the 5th hour of survival. In the three experiments referred

to above the muscles were treated with potassium at the end of the 1st hour of survival, *i.e.* while the "normal" rate of carbon dioxide production was rapidly declining; on account of the similar procedure in each case the results have been averaged and expressed as a single curve (Curve I) in Fig. 2. A comparison of these two

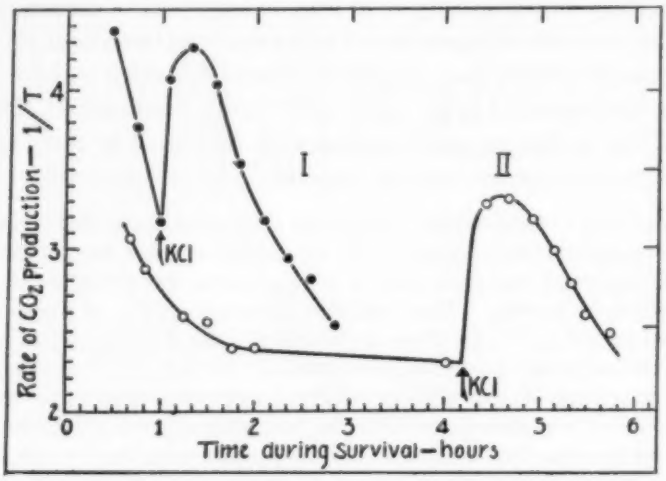


FIG. 2. The effect of potassium on the carbon dioxide production of the leg muscles of the frog.

Curve I represents the average of three experiments in which the muscles were immersed in isotonic potassium chloride solution at the end of the 1st hour of survival.

Curve II shows, in the first part of the curve, the normal rate of survival carbon dioxide production during the first 4 hours of survival; the latter part of the curve represents the effect of immersing the muscles in isotonic potassium chloride solution at the end of 4 hours and 10 minutes of survival. The data are from a single experiment.

In both cases the reciprocals of the actual times in seconds required to produce an increase in acidity from pH 7.381 to 7.168 are taken as ordinates.

curves fails to reveal any significant difference in the potassium effect, especially if it is borne in mind that the "normal" rate of carbon dioxide production during the first 4 hours of survival would, in every case, follow approximately the same course as the first part of Curve II. That is, in both cases the increased rate of carbon dioxide production is similar in magnitude and is maintained for approximately

the same length of time above what would have been the "normal" rate in Ringer solution. The significance of this similarity in the two cases will appear in what follows.

The evidence of the two preceding series of experiments shows that under the influence of potassium, in the concentration used in this work, the total acid production and the carbon dioxide production of surviving skeletal muscles of the frog are increased. The magnitude and duration of this increase in both cases correspond sufficiently closely to justify the inference that carbon dioxide may be the principal if not the only acid involved in the reaction. Granting this, it is probable that the figures in regard to carbon dioxide production do not give as true a picture of the real magnitude and time relations of the potassium effect as the data in regard to the total acid production. For the latter were obtained with single sartorius muscles, with which the rate of diffusion of the potassium ion would not be so significant a factor. The data in regard to carbon dioxide production, on the other hand, represent, at any given time, the average effect on all of the leg muscles—both large and small—so that differences in the rate of penetration of the potassium ion might be important. The justification of the method used in the measurement of the rate of carbon dioxide production lay in the necessity of using a relatively large mass of tissue in order that the carbon dioxide production would be great enough for rapid determination with the apparatus used.

The question of most interest in connection with these results is whether the increased acid production is an indirect effect of the contraction which the potassium also causes, or whether potassium acts more directly on the fundamental metabolic processes in the muscle. Experiments are being planned to test this point. But in this connection it is worth while calling attention to Fletcher's conclusions in regard to the effect of electrically stimulated contractions on the carbon dioxide production of surviving muscles.

He says, "The survival discharge of CO_2 from an excised muscle is increased during periods of contraction in the presence of abundant oxygen, the increase being proportionate, or roughly so, to the number and degree of the contractions. In agreement with observations already published, this additional yield of CO_2 accompanying contraction is absent or incomplete in the case of a muscle made to

contract in air or in nitrogen."[2] The preceding observations here referred to, were to the effect "that, under suitable conditions, the occurrence of active contractions in an excised muscle is not accompanied by an increase in the rate at which carbon dioxide is yielded by the muscle. The conditions are fulfilled when the stimulation causing contraction is not so severe as to produce marked fatigue and permanent shortening, and when it is applied during an early survival period. Stimulation during a late period may cause a rise in the rate of carbon dioxide discharge, even though the contractions evolved are much feebler than previous ones not accompanied by a rise."[3]

The bearing of these observations on the results recorded in this paper is sufficiently obvious when the following facts are kept in mind. In the first place, the increased carbon dioxide production caused by potassium seems to be the same in both early and late periods of survival (see Fig. 2); secondly, in this work the muscles were supplied only with air and probably insufficiently with that, because they were immersed throughout the experiments in solutions (Ringer and KCl) that no precaution was taken to keep well aerated; and finally, as is well known, the potassium contraction is not a permanent shortening but is very brief; tests carried out under the conditions of these experiments showed that it had a duration of only 2 to 3 minutes with sartorius muscles. No attempt is being made to suggest that the acid production and contraction are not related; but until further work can be done, these considerations, together with those to be mentioned in the following section, indicate that potassium does affect, directly, the tissue metabolism.

II.

The Effect of Potassium on the Total Acid Production of Surviving Cardiac Muscle.

Whereas potassium causes a brief contraction of skeletal muscle, its immediate effect on cardiac muscle is well known to be exactly the opposite in nature. A comparison of its effect on the metabolism of both tissues is, therefore, of interest.

The acid production of cardiac muscle was determined in the same manner and using the same precautions as described in the

[2] Fletcher (7), p. 497.

[3] Fletcher (6), p. 79.

first section of this paper regarding the acid production of skeletal muscle.

After killing a frog, the heart, including the sinus, was quickly removed and placed in a bath of Ringer solution; the auricles and ventricle were slit open in order that no solution could be carried over from one test-tube to another, inside the cavities. Between each determination the heart was freed as much as possible from all adhering solution by contact with filter paper. The whole heart was used, on account of its small size, rather than ventricular or auricular tissue alone, in order that the acid production would be great enough for rapid determination with the method used.

In all but two of the experiments nothing was done to prevent the beating of the heart during the preliminary determinations of the rate of acid production in Ringer; in fact, as was mentioned above, the sinus was left intact to facilitate this, in order to have something by which to judge the functional integrity of the tissue when treated with potassium. This might be thought, however, to invalidate the comparison of the effect of potassium on this tissue with the effect produced on inactive skeletal muscle. So in two of the experiments the hearts were rendered quiescent at the beginning by cutting off the sinus and it was observed that there was no resumption of beating during the preliminary determinations in Ringer. A comparison of the results of these two experiments with the others, indicated that this was not a vital matter. Another experiment, also interesting in this connection, was one performed with a strip of turtle ventricle, which although beating slightly at the time it was treated with potassium, was not beating either as rapidly or as regularly as the intact frog hearts. And this experiment concurred with the two mentioned above in indicating that the effect of potassium on acid production is not dependent on the simultaneous inhibition of contraction. Further considerations will be mentioned later which also tend to the same conclusion.

Altogether nineteen experiments were performed, including the one with the ventricular strip of the turtle; and, with the exception of a few determinations that were within the limit of error of the method, the rate of acid production was found to diminish progressively after treatment with potassium. Unfortunately, nothing seems

to be known as to the normal course of acid production by surviving heart muscle; but on the assumption that it might be similar to the course of survival carbon dioxide production in skeletal muscle (6) (see also Fig. 2), it became apparent that it would have to be determined with some accuracy before the potassium effect could be evaluated. Six experiments of this series that were carried out in such a way as to throw light on this question furnished the data which are represented graphically in Fig. 3. Precautions were observed which make these six experiments quantitatively comparable; they were all carried out at a temperature of 19°–20°C.; in each case the hearts were weighed so as to be able to compute and compare the rates of acid production per gm. of tissue; and finally, the experiments were run in pairs so that one could serve as a control for another —that is, two frogs were killed at the same time, their hearts removed, and determinations of the rate of acid production of each made alternately so that one might be serving as a control in Ringer solution at the time that the other was being subjected to potassium. Thus (Fig. 3) Curve I (or to be more exact, the heart which furnished the data shown in Curve I) served as a control for one of the experiments incorporated in Curve III; Curve II served as a control for another of the experiments incorporated in Curve III; and the third experiment of Curve III served as control for Curve IV. The potassium effect shown at the end of Curve II was not controlled, except by the careful determination of the preceding normal rate in Ringer, but the result is unmistakable.

Curves I and II and the first part of Curve III show that the rate of survival acid production by cardiac tissue is similar to the course of survival carbon dioxide production, as we know it for skeletal muscle; that is, the rate diminishes rapidly during the first 2 hours of survival and then continuously but more gradually therafter—at least to the 8th hour of survival, which is as long as any of these experiments were followed. These experiments also show that potassium causes an abrupt and marked diminution in the rate of survival acid production. Curve IV illustrates the necessity of such controls as were used, especially if the potassium effect is to be tested and evaluated during the first 2 hours of survival. Most of the experiments of this series were of this kind, *i.e.* the hearts

were treated with potassium after two or three "normal" determinations had been made in Ringer. And until the evidence of Curves I, II, and III was at hand there was no way of appreciating the magnitude of the potassium effect.

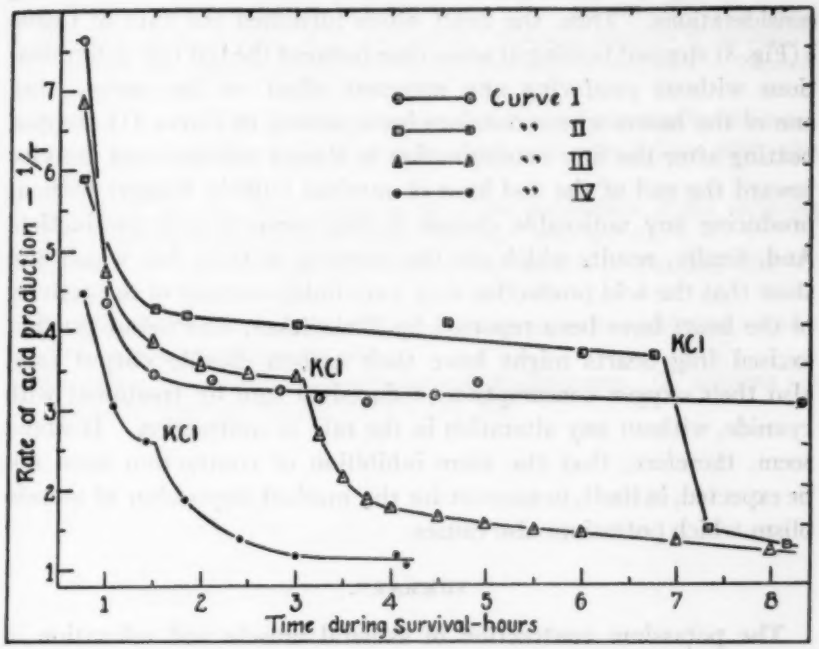


FIG. 3. The effect of potassium on the total acid production of surviving cardiac muscle of the frog.

Curve I represents the normal rate of acid production of an isolated, surviving frog heart in Ringer solution.

The other curves show the effect of potassium chloride on the acid production of surviving frog hearts after varying preliminary periods in Ringer.

Curve II shows a single experiment in which the heart was treated with potassium at the beginning of the 8th hour of survival.

Curve III shows the average of three experiments in which the hearts were treated with potassium at the end of the 3rd hour of survival.

Curve IV represents a single experiment in which the heart was treated with potassium during the 2nd hour of survival.

In each case the ordinates represent the reciprocals of the time required for *1 gm.* of tissue to produce an increase in acidity from pH 7.381 to 7.168.

Again the question arises as to whether this depression in acid production is due to the cessation of contraction which the potassium also causes or whether the effect is primarily on the metabolic processes within the tissue. Some evidence has already been advanced in favor of the latter assumption and it is strengthened by the following considerations. Thus, the heart which furnished the data of Curve I (Fig. 3) stopped beating at some time between the last two determinations without producing any apparent effect on the curve; also, one of the hearts whose data are incorporated in Curve III stopped beating after the first determination in Ringer and resumed activity toward the end of the 2nd hour of survival (still in Ringer) without producing any noticeable change in the curve of acid production. And, finally, results which are the converse of these but which also show that the acid production may vary independently of the activity of the heart have been reported by Weizsäcker, who found (8) that excised frog hearts might have their carbon dioxide output (and also their oxygen consumption) reduced to zero by treatment with cyanide, without any alteration in the rate of contraction. It would seem, therefore, that the mere inhibition of contraction need not be expected, in itself, to account for the marked depression of metabolism which potassium also causes.

SUMMARY.

The potassium contraction of skeletal muscle and relaxation of cardiac muscle have been correlated with the carbon dioxide and total acid production of these tissues.

1. The immersion of surviving sartorius muscles of the frog in isotonic potassium chloride solution causes a marked increase in the rate of acid production.

2. It is probable that carbon dioxide is the principal acid involved in the above effect.

3. The immersion of surviving cardiac muscle of the frog in isotonic potassium chloride solution causes a pronounced depression in the rate of survival acid production.

4. Reasons are given for believing that these changes in metabolism may be independent of the stimulation and inhibition of contraction which potassium simultaneously produces in these tissues.

BIBLIOGRAPHY.

1. A review of the previous work is given by Osterhout, W. J. V., Comparative studies on respiration. VII. Respiration and antagonism, *J. Gen. Physiol.*, 1919–20, ii, 1.
2. Brooks, M. M., Comparative studies on respiration. VIII. The respiration of *Bacillus subtilis* in relation to antagonism, *J. Gen. Physiol.*, 1919–20, ii, 5.
3. Guenther, A. E., A study of the comparative effects of solutions of potassium, sodium, and calcium chlorides on skeletal and heart muscle, *Am. J. Physiol.*, 1905, xiv, 73.
4. Haas, A. R., A simple and rapid method of studying respiration by the detection of exceedingly minute quantities of carbon dioxide, *Science*, 1916, xliv, 105.
5. Griffith, F. R., Jr., Does adrenalin affect the metabolism of the surviving skeletal muscles of the frog? *Am. J. Physiol.*, 1923, lxv, 15.
6. Fletcher, W. M., The survival respiration of muscle, *J. Physiol.*, 1898–99, xxiii, 10.
7. Fletcher, W. M., The relation of oxygen to the survival metabolism of muscle, *J. Physiol.*, 1902, xxviii, 474.
8. Weizsäcker, V., Arbeit und Gaswechsel am Froschherzen. II. Wirkung des Cyanids, *Arch. ges. Physiol.*, 1912, cxlvii, 135.

THE ALKALINE ISOPOTENTIAL POINT OF THE BACTERIAL CELL.*

By C.-E. A. WINSLOW and H. J. SHAUGHNESSY.

(From the Department of Public Health, Yale School of Medicine, New Haven.)

(Received for publication, May 3, 1924.)

In an earlier contribution from this laboratory (1) it was shown that the cells of *Bacillus cereus* are normally negatively charged, with an isoelectric point at about pH 3.0; that at higher pH values the velocity increases with increasing alkalinity up to about pH 10.0; and that at still higher alkalinity the velocity of migration towards the anode decreases again, apparently falling toward another isopotential point at pH 12.0 or over. In individual instances the cells lost their velocity entirely but at these high alkalinities the results were highly variable.

We have found in more recent work that the reason for this irregularity of results lies in the tendency of the bacteria to buffer the adjacent medium and create in the immediate vicinity of each cell a zone having a pH much below that of the alkaline menstruum in which they are suspended. This general phenomenon of local buffer action has been studied by Winslow and Falk (2) and its relation to the problem now in hand was revealed by the fact that when solutions of high alkalinity were used the cells would often show considerable velocity at first (while they were really exposed to a solution less alkaline than the general medium) and would gradually slow down as their buffering power became exhausted. We found that this difficulty could easily be overcome and very uniform results reached by shaking the suspension for 5 minutes before placing it in the electrophoresis cell. The pH of the suspension as a whole was checked by duplicate determinations on the final effluent liquid and was not lowered more than one-tenth of a pH unit.

* Studies here reported were aided by a grant from the Loomis Research Fund of the Yale School of Medicine.

The general apparatus and methods used were the same as those described in our earlier communications (1). At the high alkalinities we desired to study, colorimetric indicators of a satisfactory accuracy are not available, so that all pH determinations were made electrometrically, using essentially the apparatus described by Falk and Shaughnessy (3).

The results of five experiments given in Table I indicate that there really is a clearly marked and definite isopotential point at pH 13.3 and 13.4 and that beyond this point the electrical state of the cells is reversed so that they bear a distinct positive charge which increases with increasing alkalinity.

We have summarized one complete curve for *Bacillus cereus* in Table II. The figures for pH values below 12.0 are based on last year's experiments but differ slightly from those previously published (1) for the following reason. In our earlier tabulations we followed what has, we believe, been the general procedure of averaging observed times taken to cover a given distance at the three levels of the cell and then converting the average times into velocities. It is logically and mathematically somewhat sounder to convert time observations at each level into velocities and then average the three velocities. This method, which gives somewhat different final values, has been used in preparing Table II.

There is obviously a reasonably smooth curve with isopotential points just below pH 3.0 and 13.5, a zone of maximum negative charge at pH 9.0 to 11.0 and a positive charge below pH 2.0 and above pH 13.5.

In order to test the generality of this phenomenon we made five series of tests on *Bacterium coli*, the results of which are presented in Table III.

The results are very similar to those presented in Table I except that the isopotential zone is a little further to the alkaline side (*Bacillus cereus*, 13.3 to 13.4; *Bacterium coli*, 13.6 to 13.8). The enormous positive charge at pH 14.2 is especially to be noted.

It is important to know whether the positive charge observed above pH 13.5 is reversible (as we have previously shown is the case with the positive charge below pH 2.0) or whether the alkaline reversal is accompanied by irreversible chemical changes. We have tested this important point with the following results.

TABLE I.

Observed Electrophoretic Velocities (in Micra Per Second) of Cells of Bacillus cereus in Extremely Alkaline Solution.

pH.....	12.0	12.1	12.2	12.3	12.4	12.5	12.6	12.7	12.8	12.9	13.0	13.1	13.2	13.3	13.4	13.5	13.6	13.7	13.8
Exp. 1								−4.9					−4.8		0.0				
2													−3.5		0.0	+2.4			+14.8
3								−4.4					−3.6		0.0				
4													−9.4	0.0	0.0		+6.8		
5	−7.0	−7.3	−5.8		−6.3														

− indicates motion toward anode.
\+ " " " cathode.

TABLE II.

Observed Electrophoretic Velocities (in Micra Per Second) of Cells of Bacillus cereus at Different Hydrogen Ion Ranges.

pH.....	1.0–1.9	2.0–2.9	3.0–3.9	4.0–4.9	5.0–5.9	6.0–6.9	7.0–7.9	8.0–8.9	9.0–9.9	10.0–10.9	11.0–11.9	12.0–12.9	13.0–13.5	13.6–13.9
No. of observations.....	8	8	8	3	7	13	5	8	5	11	8	6	10	2
Average velocity.....	+0.4	−0.1	−2.6	−9.7	−12.9	−9.9	−15.2	−15.3	−18.6	−18.8	−7.3	−5.9	−1.9	+10.8

TABLE III.

Observed Electrophoretic Velocities (in Micra Per Second) of Cells of Bacterium coli in Extremely Alkaline Solution.

pH.....	12.7	12.8	12.9	13.0	13.1	13.2	13.3	13.4	13.5	13.6	13.7	13.8	13.9	14.0	14.1	14.2
Exp. 1					−5.7		−1.9			0.0						
2	−4.7			−5.2		−2.4										
3							−1.5			0.0				0.0		
4						−2.0				0.0			+15.1			+34.4
5									−1.0	0.0		0.0		+12.3		

At pH 13.8 a culture of *Bacillus cereus* showed a velocity of 9.8 micra per second toward the cathode. When readjusted by the addition of normal HCl to pH 6.6, the migration was toward the anode with a velocity of 5.2 micra. In a second experiment the cells moved toward the cathode with a velocity of 13.5 micra at pH 14.1 and an adjustment to pH 9.8 moved toward the anode with a velocity of 4.1 micra. These negative velocities after readjustment from an extreme alkaline reaction are, it will be noted, much less than those recorded for pH values of 6.6 and 9.8 in Table II. This we believe however to be due to the presence of the salt formed in neutralizing the high alkalinity. The final salt concentration was in the neighborhood of a 0.5 molar solution of NaCl which we have previously shown (1) gives a velocity of just about 5 micra with *Bacillus cereus*.

CONCLUSIONS.

Irregularities in migration velocity of bacterial cells in the highly alkaline solutions are due to the buffering effect of the cells upon the immediately adjacent zone of menstruum. Consistent results can be obtained by shaking the suspension thoroughly before placing it in the electrophoretic cell.

When observed in this way both *Bacillus cereus* and *Bacterium coli* show an isopotential point near pH 13.5, that for *Bacillus cereus* being slightly below, and that for *Bacterium coli* slightly above this point. At more alkaline reactions the cells acquire a positive charge which increases with further increase in pH to very high values.

BIBLIOGRAPHY.

1. Winslow, C.-E. A., Falk, I. S., and Caulfield, M. F., 1923–24, Electrophoresis of bacteria as influenced by hydrogen ion concentration and the presence of sodium and calcium salts, *J. Gen. Physiol.*, vi, 177.
2. Winslow, C.-E. A., and Falk, I. S., 1923, Studies on salt action. VIII. The influence of calcium and sodium salts at various hydrogen ion concentration upon the viability of *Bacterium coli*, *J. Bact.*, viii, 215.
3. Falk, I. S., and Shaughnessy, H. J., 1922–23, Effect of certain electrolytes on the buffering power of *Bacterium coli*, *Proc. Soc. Exp. Biol. and Med.*, xx, 426.

A NOTE ON THE RELATIVE PHOTOSENSORY EFFECT OF POLARIZED LIGHT.

By W. J. CROZIER* and A. F. MANGELSDORF.

(*From the Zoological Laboratory, Rutgers College, New Brunswick.*)

(Received for publication, May 22, 1924.)

I.

Although several considerations lead to the suspicion that an investigation of the stimulating power of polarized light may give useful results, the question seems not to have been put to test. Such a test was planned some years ago when it was realized that in devices employed for study of animal responses controlled by light there is often opportunity for polarization by reflection. The matter is of interest also in connection with the action of moonlight (Semmens, 1923). But that the polarization of moonlight, often slight, is responsible for any pronounced biological effect is still distinctly an open matter (for comment on this point compare Fox, 1923-24). The same must be said for a not inconceivable effect of polarization in diffused light under the surface of ocean water as noted by Brooks (1922). The light produced by such luminous animals as *Mnemiopsis* is unpolarized.

The question is more significant in relation to the possible rôle in photic stimulation of fluorescent substances in the definitely oriented photoreceptive cells of arthropods. Weigert (1920) discovered the polarization of fluorescent light from dyestuffs dissolved in a viscous medium (gelatin). Subsequent investigation (Carelli and Pringsheim, 1923) has shown that dyestuffs devoid of photoluminescence when dissolved in non-viscous media may in a viscous medium show polarized fluorescence when excited by polarized light. This may be understood as resulting from the definite and constrained orientation of anisotropic fluorescing molecules. It may be suggested that a constrained

* Research Associate, Carnegie Institution of Washington.

orientation should be conceivable in surface films (Langmuir, 1917; Adam, 1922) of one kind or another, as well as in an extremely viscous solvent.

We have made three groups of experiments designed to compare (1) the activating effects of light with different planes of polarization and (2) the relative stimulating efficiencies of polarized and non-polarized lights. The general method in the first two experiments was to record the trail pursued by an organism when illuminated from either side by lights of equal intensity. As shown by Patten (1914) this method is one of very considerable accuracy, with a suitable animal. It is to be expected that a bilaterally symmetrical and negatively phototropic organism, unless its receptor surfaces are effectively parallel (Crozier, 1917), will move in a line perpendicular to the opposed paths of two equally intense beams of light; under certain conditions positively phototropic organisms may also exhibit this behavior. A series of unpublished results obtained by one of us in collaboration with Dr. W. H. Cole shows that the observed mean angular deflection of blow-fly larvæ under a given condition of bilateral illumination is subject to a probable error of about 6 per cent of the mean.

II.

Preliminary trials with blow-fly larvæ at about the age of their maximum sensitivity to light (4 days; Patten, 1916) showed no obvious difference between the effects of polarized and of non-polarized lights of equal intensities (35 m.c.).

We then employed the negatively phototropic land isopod *Cylisticus convexus*. After about 2 hours dark adaptation individual isopods were allowed to creep in a field of opposed beams of polarized light of equal intensity, the plane of polarization of one beam being vertical and of the other beam, horizontal. Polarization was secured by means of two large Nicol prisms. The sources of light were two similar 100 watt tungsten filaments in nitrogen filled bulbs. The intensities (about 45 m.c.) were equalized by means of a Lummer-Brodhun photometer. The isopods crept upon moist filter paper spread upon a large sheet of glass which had been ruled with dark blue coordinate lines. These lines, visible through the moist filter

paper, formed a reference grid permitting accurate mapping of the path followed by each isopod upon similarly marked paper.

As found by Patten (1914) with the blow-fly larva and as has been observed with other animals, there is often evidenced a more or less pronounced asymmetry of response. This might be due either to asymmetrical fluctuating variations of peripheral sensitivity, to central conditions affecting crossed transmission tracts, or to differences in the musculature. At all events, its effects upon the trail pursued under bilateral illumination are readily discounted by means of two trails measured for each individual creeping in respectively reversed directions. The isopods were first headed (in the dark) toward source *A*, then in second trial toward source *B*. Light from both sources being admitted simultaneously, the organism swung about to a path almost perpendicular to the line connecting the two lights of equal intensity. Light from source *A* had vertical plane of polarization; that from *B*, horizontal.

The angle of orientation was measured by means of a 9 cm. protractor. The mean deflection, in forty-four trails of twenty-two isopods, was 68.13°. The failure to assume a path at right angles to the line connecting the sources of light is probably determined by the convexity of the receptive surfaces of the eyes, their obtuse angle with respect to one another, and the shading effect of the carapace. This view is supported by the fact that with lights of higher intensity an angle of more nearly 90° is observed.

If deflections toward light *A* be termed +, those toward *B*, −, a superior stimulating effect of light with one particular plane of polarization should result in a difference between the two mean deflections. But the mean + deflection was 68.02° ± 2.40°, the mean − deflection 68.28° ± 2.92°. Calculating the ratios between + and − deflections for each isopod, and averaging the deflections, we find that the mean ratio is as 1 to 1.034, the probable error of the mean ratio being ± 0.035.

A second set of experiments with intensity about 90 m.c. gave the following result.

Average deflection	83.20°
Average + deflection	83.75° ± 3.37°
Average − deflection	82.66° ± 3.46°

Mean ratio of + : −, 1:1.031° ± 0.052°.

Clearly, no real difference in stimulating power is discernable in relation to the position of plane of polarization. Since the lights were equalized by visual photometry, it should be pointed out that there is no polarization effect of sensible magnitude in the human eye.

III.

The second series of experiments involved comparing the stimulating values of polarized and non-polarized lights, by the method already described. The animal used was the coral red milkweed beetle, *Tetraopes tetraopthalmus*. Freshly collected animals were allowed to creep in a field of balanced illumination, and the trails recorded.

Since these beetles are *positively* phototropic, it is necessary to consider the theory of the orientation process. It cannot be assumed that in a field of unequally stimulating opposed light beams, a positively heliotropic organism will necessarily orient itself after the fashion of a blow-fly larva. In case the two lights are of unequal effectiveness, the positively phototropic animal would be expected to move toward the more effective light; there is no opportunity to equalize illumination of the two eyes by altering the position of the body axis (Moore, 1923-24). *Vanessa* caterpillars do precisely this (von Buddenbrock, 1917). But the notion, supposedly justified by this fact, that a *negatively* phototropic organism bisects the field between opposed lights because it "seeks out the dark" (Bierens de Haan, 1921) quite fails to explain the relation between the actual angle of path and the ratio between the light intensities (Patten, 1914; Northrop and Loeb, 1922-23). Even with positively phototropic organisms, provided the rate of locomotion be sufficiently high and sideward movements of the head not pronounced, it is to be expected that in the field of almost equally intense opposed lights a path will be followed at an angle of about 90° to that connecting the two sources. Our experience with *Tetraopes* shows this to be the case for the milkweed beetle (*cf.* also Mast and Dolley, 1924).

The results of four series of tests with *Tetraopes* are summarized in the following table; in Sets 2 and 4 the lights were about 50 per cent more intense than in Sets 1 and 3.

Set.	No. of beetles (two trails each).	Mean deflection toward non-polarized light.	Plane of polarization.
1	30	5.12° ± 1.32°	Horizontal.
2	13	6.13° ± 2.52°	"
3	30	4.14° ± 2.12°	Vertical.
4	16	1.29° ± 1.05°	"

In spite of a certain suggestion of uniformity, the differences are within the limit of random error of the observations (differences from 0 angle being less than three or four times the probable error), and no definite evidence is given as to any sensible variations in stimulating power correlated with polarization. Since non-polarized radiation, not passing through a Nicol prism, might conceivably be of effectively different quality than the polarized, a real difference due to polarization would be expected to manifest itself in the relative influences of vertically and horizontally polarized beams.

IV.

A different method of contrasting the effects of horizontally and of vertically polarized light is possible with an animal in which phototropism may be made to counterbalance and to overcome another tropistic response. The larvæ of the beetle *Tenebrio* permit such an experiment (Crozier, 1923-24). Light of adequate intensity falling laterally upon a larva through a vertical glass plate with which the larva creeps in contact, forces orientation away from the glass surface. A definite amount of light (about 134 m.c.) is required to overcome positive stereotropism.

With an optical arrangement designed to give a fairly intense beam of nearly parallel polarized light, the mean critical distances from the source affording just enough light to suppress stereotropism were as follows in two sets of experiments, (*a*) and (*b*).

Experiment.	Results.	
	m.m.	
(a)	158	Polarization vertical.
	152	" horizontal.
(b)	150	" vertical.
	153	" horizontal.

The differences are of no significance.

V.

The negative outcome of the experiments does not of course preclude discovery in other animals of the effect sought. Although fluorescent materials as sensitizers in sense organs have been referred to as a source of photic excitation (*cf.* Demoll, 1910; Dubois, 1914), it happens that in at least one case (Crozier, 1920–21) light passed through a fluorescing extract of a skin pigment seems unimpared in its stimulating power for a photosensitive integument. Similar relations obtain in connection with the photodynamic action of fluorescent dyestuffs (Clark, 1918-19). It may prove that in more viscous protoplasmic medium a more intense fluorescence is possible. The arrangement of retinular elements in radial symmetry in each ommatidium of the arthropod eye might be appealed to in explaining the lack of observable specific influence of the plane of polarization.

SUMMARY.

Experiments were made to compare the stimulating effectiveness of vertically and horizontally polarized lights and non-polarized lights of equal intensity upon phototropic movements of the beetle *Tetraopes tetraopthalmus*; and to compare the effectiveness of two light beams polarized at right angles to one another upon phototropic orientation of the land isopod *Cylisticus convexus*. *Tetraopes* is positively, and *Cylisticus*, negatively phototropic. Tests were also made of the intensities of horizontally and of vertically polarized light required to inhibit stereotropism in larvæ of *Tenebrio*. Under the conditions of the tests, no certain qualitative effect connected with polarization could be detected.

We wish to thank Professor George Winchester, Department of Physics, Rutgers College, for the use of certain pieces of apparatus.

BIBLIOGRAPHY.

Adam, N. K., 1922, *Proc. Roy. Soc. London, Series A*, ci, 452.

Bierens de Haan, J. A., 1921, *Biol. Centr.*, xli, 395.

Brooks, E. E., 1922, *Nature*, cx, 114.

von Buddenbrock, W., 1917, *Sitzungsber. Heidelberger Akad. Wissensch., math.-naturw. Kl., 1 Abhandl*

Carelli, A., and Pringsheim, P., 1923, *Z. Physik*, xviii, 317.
Clark, J. H., 1918–19, *Am. J. Physiol.*, xlvii, 251.
Crozier, W. J., 1917, *Am. J. Physiol.*, xliii, 510; 1920–21, *J. Gen. Physiol.*, iii, 57; 1923–24, *J. Gen. Physiol.*, vi, 531, 647.
Demoll, R., 1910, *Ergebn. u. Fortschr. Zool.*, xiii, 431, 474.
Dubois, R., 1914, La vie et la lumière, Paris.
Fox, H. M., 1923–24, *Proc. Roy. Soc. London, Series B*, xcv, 523.
Langmuir, I., 1917, *J. Am. Chem. Soc.*, xxxix, 1848.
Mast, S. O., and Dolley, W. L., Jr., 1924, *Am. J. Physiol.*, lxviii, 285.
Moore, M. M., 1923–24, *J. Gen. Physiol.*, vi, 393.
Northrop, J. H., and Loeb, J., 1922–23, *J. Gen. Physiol.*, v, 581.
Patten, B. M., 1914, *J. Exp. Zool.*, xvii, 213; 1915, *Am. J. Physiol.*, xxxviii, 313; 1916, *J. Exp. Zool.*, xx, 585.
Semmens, E. S., 1923, *Nature*, cxi, 49; 1923, *Chem. and Ind.*, xlii, 954.
Weigert, F., 1920, *Z. Physik*, iii, 437; 1921, *Z. Elektrochem.*, xxvii, 481.

THE LOCOMOTION OF LIMAX.

I. Temperature Coefficient of Pedal Activity.

By W. J. CROZIER* and G. F. PILZ.

(From the Zoological Laboratory, Rutgers College, New Brunswick.)

(Received for publication, May 22, 1924.)

The creeping of such a gasteropod as *Limax maximus* results from (Fig. 1) a rhythmic succession of evenly spaced, progressive wave-like deformations of the pedal surface. It should be possible to utilize these waves for the study of neuromuscular physiology in the intact animal, avoiding in this way effects due to lack of proper circulation and the like, as when isolated organs are employed (Vlès, 1908; Crozier, 1922–23).

The pedal activities of a number of chitons and gasteropods have been described and classified in a comparative way (Vlès, 1907, 1913; Parker, 1911, 1914; Olmsted, 1917–18; Crozier, 1918–19). Experiments by Biedermann (1905), Künkel (1903), Bethe (1903), Arey and Crozier (1919), and ten Cate (1923) agree in pointing to the fact that the formation of the pedal waves and their procession over the foot depend essentially upon the intrinsic nervous mechanism of this organ, and presumably upon its nerve net. The mechanism of creeping is normally released, reflexly, by impulses traveling over the central ganglia and pedal nerves.

The neuromuscular creeping organ of *Limax* is definitely polarized, as is true of the majority of gasteropods; the pedal waves are *direct*, coursing from posterior to anterior end of the foot. The waves occupy the median third of the foot. About eleven to nineteen waves are present at one time, depending chiefly upon the size of the animal, although in very rapid creeping the number may be increased. Each wave is a region of the creeping surface lifted free from the substratum; within this temporarily lifted zone the contractile elements of

* Working with the support of the Carnegie Institution of Washington.

the foot serve to move the pedal substance forward. This conception of the pedal wave (Parker, 1911; Olmsted, 1917–18) has been questioned by certain observers (van Rijnberk, 1918–19; ten Cate, 1923). Our observations on *Limax* agree with those of Parker (1911). The correctness of this general interpretation is supported by the results of inspecting cases where the pedal wave is of gross dimensions, not only in mollusks (Parker, 1914, 1917–18, 1921) but also in representatives of other phyla practicing essentially this method of locomotion (*Metridium*, Parker, 1917; larvæ of slug-moths, Crozier, 1923–24).

FIG. 1. *Limax maximus*, ventral view showing character of pedal waves.

The precise negative geotropism of *Limax* (Davenport and Perkins, 1897) makes it easy to study the creeping of the slug under conditions such that the rate of doing work (lifting the body vertically) varies directly with the animal's weight and with the velocity of upward movement. A slug is placed at the bottom of a large cylindrical glass jar about 3 feet high, the inside of the jar being previously moistened by rinsing with tap water. On beginning movement, the wall of the jar is quickly encountered and the animal in practically every instance orients in such fashion as to move upward in a straight line on the wall

of the cylinder. The jar is covered to exclude air currents, which may otherwise result in irregularities of creeping. A thermometer in the jar, suspended from the cover, gives the temperature.

In our measurements we have taken account of the following: (1) the weight of the individual, (2) the dimensions of the pedal surface, (3) the speed of creeping (V), (4) the frequency of pedal waves (F), (5) the upward movement due to a single wave (A), (6) the velocity with which a single pedal wave traverses the foot (v), and (7) the

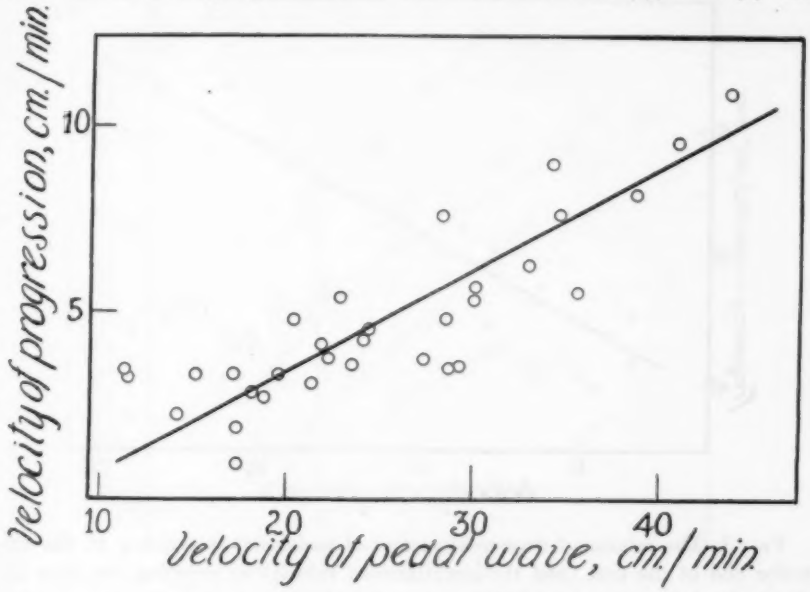


FIG. 2. The relation between velocity of a single pedal wave and the simultaneous velocity of upward creeping.

temperature. Since (3), (4), and (6) are not independent quantities, and since the speed of upward movement varies from moment to moment, the observations (particularly as to (3), (4), and (5)) must be made concurrently during brief intervals. This is accomplished by noting the time and the number of pedal waves required to move the animal upward by a known distance (usually 1 to 2 cm.) marked upon the surface of the glass jar. The temperature was varied between 11° and 28°C.; these represent about the extreme limits for active creeping. The adhesive power of the foot depends largely upon its

extruded mucus, and this may constitute a slight error in comparing the effectiveness of the pedal mechanism at different temperatures, since at lower temperatures the animal is doing work against the fixation due to a less fluid slime. Other sources of variation greatly outweight this one, however. The results described are based upon series of readings with each of thirty-four *Limax maximus* varying in weight from 0.18 to 15.2 gm.

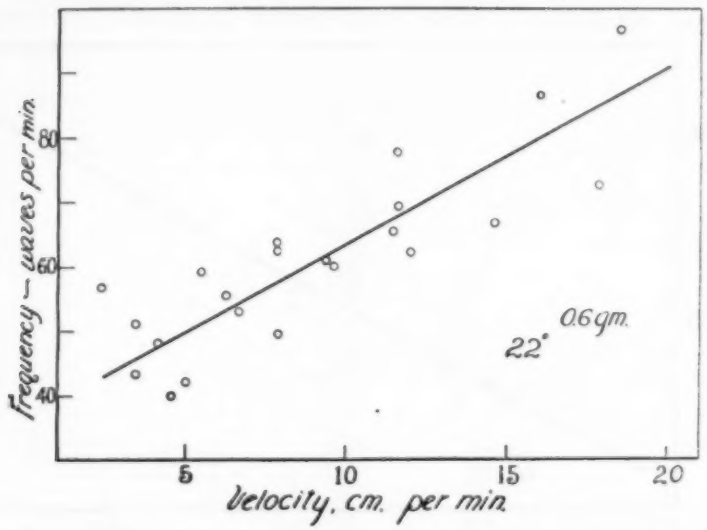


FIG. 3. The relation between frequency of pedal waves arriving at the anterior end of the foot, and the simultaneous velocity of creeping, for four individuals of the same weight (0.6 gm.) and at the same temperature.

The velocity of a single pedal wave is directly proportional to the speed of upward creeping (Fig. 2). Variations from strictest proportion unquestionably arise in large part from the complex character of the pedal musculature; a deficiency in wave frequency may thus be compensated by greater dimensions of the single waves. But in general trend the proportionality of v to V is unmistakable, and in spite of the complex organization of the foot, and of the considerable likelihood of slight observational error, seems to hold regardless of the weight of the animal, or of the temperature. The actual magnitudes of v are of the order associated with other measurements of

(presumably) nerve net-conducted processes (Parker, 1918–19), but this correspondence is not in itself conclusive evidence of nerve net transmission (Crozier, 1923–24).

The velocity of progression is also directly proportional to the number of pedal waves per minute passing a given point on the foot (F). Data in Fig. 3 were obtained from tests with four animals

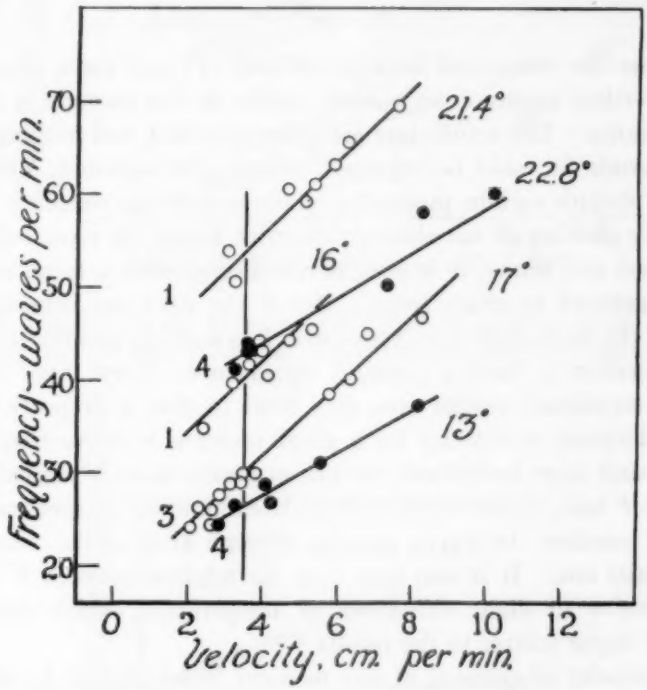


FIG. 4. The relation between wave frequency and velocity of progression is illustrated for different individuals (numbered 1, 3, 4) and at different temperatures. The slope of the lines for *Limax* 4 is unusual, but consistent at different temperatures.

of identical weights (0.6 gm.). In general, this type of relation is found, and with the same factor or proportionality (slope), when observations pertaining to a single individual are considered. The position of the line (Figs. 3, 4) is shifted toward the V axis at lower temperatures, but the slope of the line remains constant. Occasionally an exceptional individual is found for which the slope of the line con-

necting V with F is different; the same slope is retained in the observations from such an individual at a different temperature (Fig. 4).

Vlès and his collaborators (Vlès, 1908; Peyrega and Vlès, 1913; Vlès and Bathellier, 1920) have studied the relations between pedal waves and locomotor efficiency in gasteropods. Vlès and Bathellier (1920) propose an equation of the form

$$V = Ae^{BF}$$

to express the connection between velocity (V) and wave frequency (F) in vertical ascension by various snails; in this formula A and B are constants. The actual data are quite scattered, and nothing more than a crude fit could be expected. From this equation, Vlès proposes to deduce certain properties of the contractile elements in the foot. By plotting all our observations with *Limax* (at room temperature) upon one frame, it is possible to observe such a trend as Vlès has endeavored to characterize. But if the data are considered in terms of the single individuals involved, it is scarcely possible to regard Vlès' equation as having physical significance. Very large *Limax*, and an occasional smaller one, may tend to give a proportionately greater increase in velocity for a given increase in wave frequency; and for such large individuals the line of observations is shifted away from the F axis, so that when readings from numbers of specimens are lumped together the curve passing through their midst seems an exponential one. It is also true that the relation between V and F is responsive to slight differences of temperature, which assists in giving a vague scatter to the results.

The velocity of creeping at any moment is determined by the frequency of the waves and by the advance due to a single wave; the latter is dependent upon the interplay of two general sets of muscle fibers in the foot, respectively oriented dorsoventrally and longitudinally (Car, 1897). The advance due to a single wave is measured as an average from the number of waves disappearing at the anterior end of the foot while the anterior border of the foot is advancing over a measured distance. This quantity, A, is of course directly proportional to the velocity of creeping, but the slope of the line of proportionality becomes greater at lower temperature (Fig. 5). At constant temperature the slope of the line for different individuals is the same

(Figs. 6, 7). In general, with animals of increasing weight the line is displaced from the axis of velocities (Fig. 7); individuals of the same weight yield points falling upon the same line (Fig. 6).

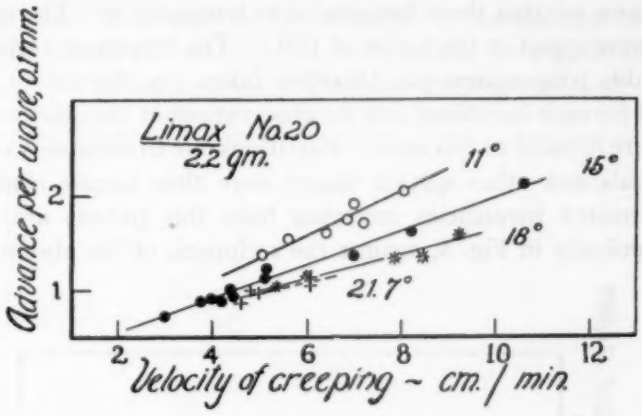


FIG. 5. The advance due to one wave is directly proportional to the velocity of creeping, but the slope of the line varies inversely with the temperature.

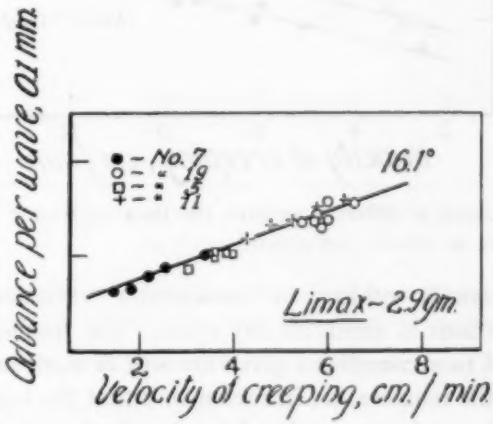


FIG. 6. The advance per wave is proportional to the velocity of creeping; the relationship is identical for animals of the same weight.

From these findings it is possible to estimate the effects of temperature upon pedal activity.

The frequencies of waves required at different temperatures to produce a given velocity of upward creeping were measured for twenty-

four animals. By interpolation upon such curves as are shown in Fig. 4, the frequency at each experimental temperature was obtained which corresponded to a velocity of 3.5 cm. per minute. Curves were drawn relating these frequencies to temperature. The separate curves overlapped in the region of 17°C. The frequency corresponding to this temperature was therefore taken (on the curve) as 100 per cent for each individual and the observations at the other temperatures were reduced to this scale. Variations due to differing weights of individuals and other specific causes were thus largely eliminated. The corrected frequencies emerging from this process are plotted logarithmically in Fig. 8, against the reciprocal of the absolute tem-

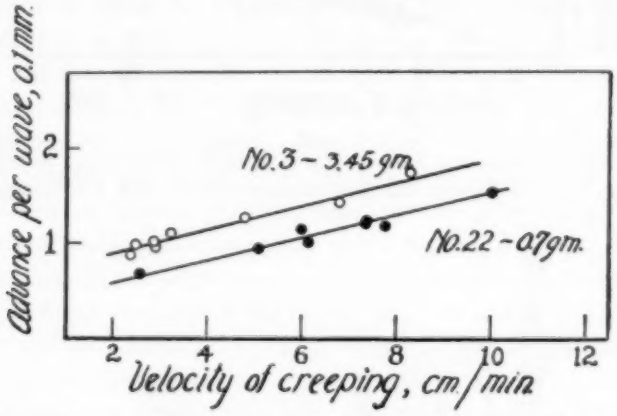


FIG. 7. For *Limax* of differing weights, the lines expressing the relation of advance per wave to velocity are parallel.

perature. Although subject to considerable variation, the trend of the observations is unmistakably clear. The frequency of pedal waves required to accomplish a given amount of work in a given time is affected by the temperature in such a way that the logarithm of the frequency is inversely proportional to the absolute temperature, as required by Arrhenius' (1889) formula. For the present case the value of μ in this formula is about 10,700 (Q_{10} for 11° to 21° = 2.1).

The velocity of the individual pedal wave (v) is the same for a given velocity of creeping (V), regardless of the temperature. Hence it is impossible to obtain a direct measure of the temperature coefficient

for the "velocity of a single wave." The constancy of v for fixed V comes about from the fact that with lower temperatures the advance due to any single wave is enhanced. This may be measured by reading from curves such as shown in Fig. 7, the increase in average "advance due to one wave" which is requisite for a given increment in velocity. This amount varies inversely with the temperature. The

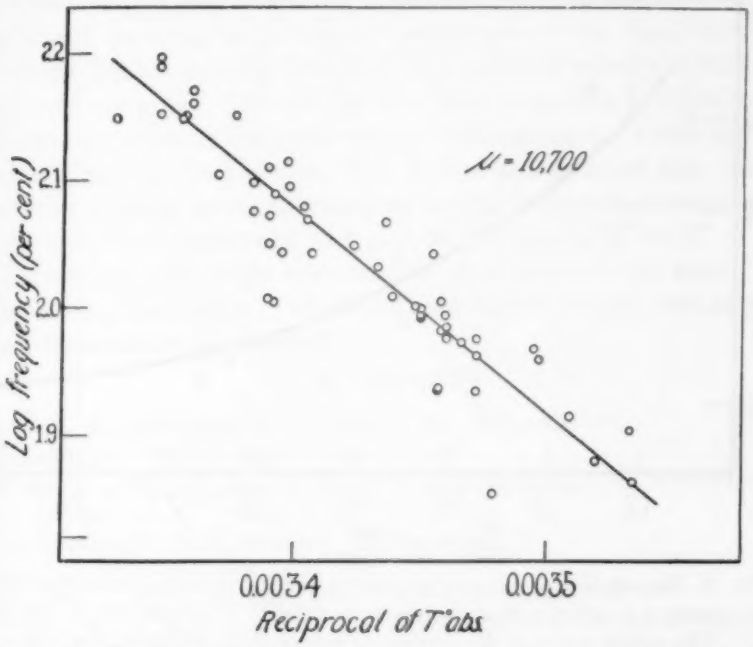


FIG. 8. The logarithm of the frequency of pedal waves required to lift the body of the animal vertically at a fixed rate is inversely proportional to the absolute temperature. The equation of the line drawn in $\log F = \frac{10{,}700}{2T} + C$, where T is the absolute temperature.

results (Fig. 9) permit only a rough estimate of the temperature coefficient, which is about $Q_{10} = -2.2$. At lower temperatures the individual wave is more effective, under conditions permitting comparison, probably due to the heightened tonus of its contractile fibers; that is, the forward movement of the temporarily lifted portion of the foot is more extensive.

Indirectly, the temperature coefficient for speed of a pedal wave (v) may be deduced from the fact that F and V are directly proportional, as also v and V, while the temperature coefficient of F is known. The coefficient for v should therefore be the same as that for F.

It is not without significance that the temperature coefficient for pedal activity in *Limax*, which may be regarded as the coefficient of

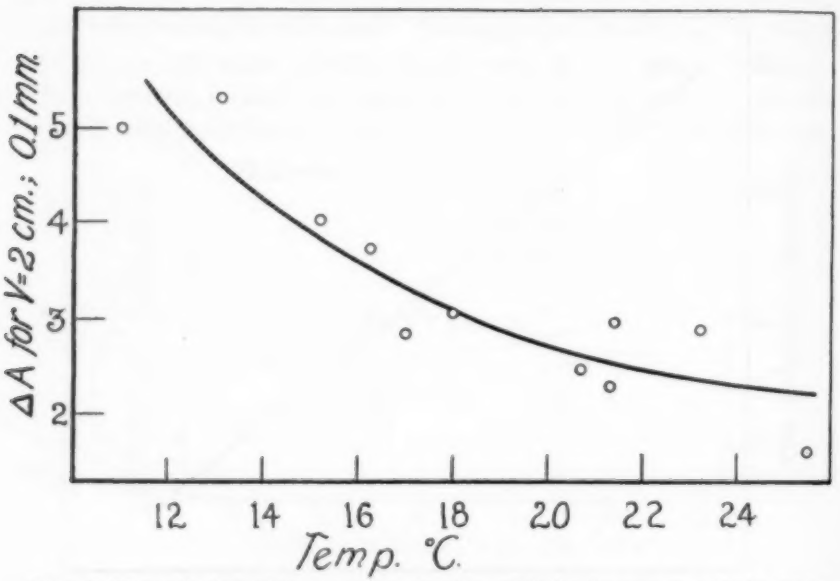


FIG. 9. The relation of temperature to the vertical advance per single wave (ΔA) required to effect a given increment in velocity of progression (2 cm. per min.). The points are each the average to two or three determinations; Q_{10} = approximately − 2.3.

nervous initiation of waves and also of their conduction, is similar to that obtained in another instance of nerve net transmission. From Parker's (1920) data giving the rate of transmission in the colonial body of *Renilla* at several temperatures I calculate a value of μ of about 11,200,[1] which is probably not significantly different from that found with *Limax*. Comparison on this basis is of interest not only

[1] This applies to the temperature interval 11° to 22.8°; from 22.8° to 31°, the value of μ is higher (16,100). The occurrence of a "break" in the temperature relation at about the temperature of the normal environment is not at all unusual in such data; it is planned in another connection to consider its significance.

because μ gives a constant covering a wide range of temperatures, which the commonly employed "Q_{10}" does not, but also because the physical meaning of μ as a "heat of activation" holds out the prospect of classifying protoplasmic processes when investigated from this standpoint.

SUMMARY.

Pedal progression of the slug *Limax maximus* was studied to obtain relations between wave velocity on the sole of the foot, wave frequency, the advance due to a single wave, and the velocity of vertically upward creeping. Each of the first three quantities is directly proportional to the simultaneous velocity of progression. Under comparable conditions, that is when work is done at a constant rate, the frequency of pedal waves is influenced by the temperature according to the equation of Arrhenius, with $\mu = 10{,}700$ (Q_{10} for 11° to 21° = 2.1). The velocity of a single wave must have very nearly the same "temperature characteristic," which is found also in another case of nerve net transmission (in *Renilla*).

BIBLIOGRAPHY.

Arey, L. B., and Crozier, W. J., 1919, *J. Exp. Zool.*, xxix, 187.

Arrhenius, S., 1889, *Z. physik. Chem.*, iv, 226.

Bethe, A., 1903, Allgemeine Anatomie und Physiologie des Nervensystems, Leipsic.

Biedermann, W., 1905, *Arch. ges. Physiol.*, cvii, 1.

Car, L., 1897, *Biol. Centr.*, xvii, 426.

ten Cate, J., 1923, *Arch. néerl. physiol. homme et animaux*, viii, 377.

Crozier, W. J., 1918–19, *J. Exp. Zool.*, xxvii, 359; 1923–24, xxxviii, 323; 1922–23, *Anat. Rec.*, xxiv, 398.

Davenport, C. B., and Perkins, H., 1897, *J. Physiol.*, xxii, 99.

Künkel, K., 1903, *Zool. Anz.*, xxvi, 560.

Olmsted, J. M. D., 1917–18, *J. Exp. Zool.*, xxiv, 223.

Parker, G. H., 1911, *J. Morphol.*, xxii, 155; 1914, *Contributions from the Bermuda Biological Station for Research, No. 31*, iii; 1917, *J. Exp. Zool.*, xxii, 111; 1917–18, *J. Exp. Zool.*, xxiv, 139; 1918–19, *J. Gen. Physiol.*, i, 231; 1920, *Proc. Am. Phil. Soc.*, lix, 171; 1921, *J. Exp. Zool.*, xxxiii. 205.

Peyrega, E., and Vlès, F., 1913, *Bull. Soc. zool. France*, xxxviii, 251.

van Rijnberk, G., 1918–19, *Arch. néerl. physiol. homme et animaux*, iii, 539.

Vlès, F., 1907, *Compt. rend. Acad.*, cxlv, 276; 1908, *Bull. Soc. zool. France*, xxxiii, 175; 1913, *Bull. Soc. zool. France*, xxxviii, 242.

Vlès, F., and Bathellier, J., 1920, *Compt. rend. Acad.*, clxxi, 1083.

THE KINETICS OF TRYPSIN DIGESTION.

V. SCHÜTZ'S RULE.

BY JOHN H. NORTHROP.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, May 6, 1924.)

The action of many enzymes is inhibited by the products of the reaction. This is particularly true of the proteolytic enzymes and is one of the causes for the decreasing monomolecular constants found under ordinary conditions. In order to study the effect of this inhibiting action of the products, it is necessary to regulate conditions so that there is no "spontaneous" inactivation of the enzyme. It is also necessary to have a relatively small amount of enzyme and a large amount of substrate, since otherwise the percentage inactivation of the enzyme will be small. Under these conditions the substrate concentration is decreasing owing to hydrolysis, and the active enzyme is decreasing owing to the combination with the products of hydrolysis. The mass action equation for the reaction is, therefore,

$$-\frac{dS}{dT} = KSQ \tag{1}$$

in which S is the substrate concentration and Q the active enzyme concentration at the time, T. If A is the concentration of substrate when $T = 0$, and x the concentration of products at any time, the reaction may be written

$$\frac{dx}{dt} = KQ(A - x) \tag{2}$$

in which Q is also a function of x. It has been found[1] that the reaction between the enzyme and the products of hydrolysis is accurately expressed by the equation

[1] Northrop, J. H., *J. Gen. Physiol.*, 1921–22, iv, 245.

$$\text{enzyme} + \text{inhibitor} \rightleftharpoons \text{enzyme} - \text{inhibitor or } \frac{Q \cdot (x - (E - Q))}{E - Q} = K$$

in which $E - Q$ is the combined, inactive enzyme. It was pointed out by Arrhenius[2] that this equation could be written

$$Q = \frac{KE}{x}$$

as soon as x becomes large with respect to E. This is a property of any similar mass action system and was verified experimentally.[1] If this value is substituted for Q, in equation (2), we obtain

$$\frac{dx}{dt} = K_A E \frac{A - x}{x} \tag{3}$$

in which K_A is a constant equal to the product of the reaction velocity constant and the dissociation constant for the enzyme-product compound. The integral of this expression (Arrhenius[2]) is

$$K_A = \frac{A \ ln \frac{A}{A - x} - x}{ET}$$

This equation assumes that the reaction rate is proportional to the concentration of substrate and inversely proportional to the concentration of the products of the reaction. It evidently will not hold at the beginning of the reaction since the amount of enzyme is not inversely proportional to the products of the reaction until this value is large with respect to E.

If the substrate concentration is high there will be a period in the reaction during which it will not change markedly. If this also is considered constant, equation (3) becomes

$$\frac{dx}{dt} = K'_S \frac{E}{x}$$

or on integration

$$K'_S = \frac{x}{\sqrt{ET}}$$

which is Schütz's rule.

[1] Arrhenius, S., *Medd. Kong. Vetsakad. Nobelinst.*, 1908, i, 1.

These equations were found by Arrhenius[2] to agree quite well with Sjöqvist's results and the experiments were repeated and confirmed by the writer. In both cases the methods used to follow the digestion (conductivity or amino acid increase) are open to the objection that the value A, the total substrate concentration, is rather uncertain, and also to the objection that the whole series of consecutive hydrolyses are followed. In the present experiments these points have been eliminated by following directly the disappearance of the protein.

Experimental Procedure.

13 gm. of casein and 4 gm. of boric acid were suspended in water and sodium hydroxide added with stirring until the solution was brought to pH 7.4. It was then cooled to zero and filtered. 2 cc. of trypsin solution were then added to 25 cc. portions of the casein solution and the flasks kept in melting ice in the ice box. 1 cc. samples were taken for analysis, diluted to 5 cc., and precipitated with 15 cc. of 3 per cent trichloroacetic acid.[3] The precipitate was washed with trichloroacetic acid, dissolved in alkali, and made up to 10 cc. Kjeldahl determinations were made on 2 cc. of this solution. The figures are the average of four determinations. Under these conditions there is practically no spontaneous inactivation of the enzyme. A heavy mass of crystals slowly separated out during the digestion.

The result of the experiment is shown in Fig. 1. Table I shows the time required to hydrolyze various amounts of protein and also the amount hydrolyzed after various times. The time required to hydrolyze an equal amount is nearly inversely proportional to the relative concentration of enzyme whereas the amount of hydrolysis at equal times is much less than the relative trypsin concentrations and is in proportion to the square root of the relative enzyme concentration during a portion of the hydrolysis. This result shows that the rate is decreasing solely as a function of the substrate concentration and not as a function of the time. This is the predicted result.

According to Schütz's rule, the amount of protein hydrolyzed, plotted against the product of the square root of the enzyme concentration into the elapsed time, should be a straight line and the curves for various enzyme concentrations should be superimposable. Fig. 2

[3] Hiller, A., and Van Slyke, D. D., *J. Biol. Chem.*, 1922, liii, 253.

shows that this is true over a part of the curve but that the observed points are below the line at the beginning and end of the experiment. The discrepancy at the beginning is due to the fact mentioned above

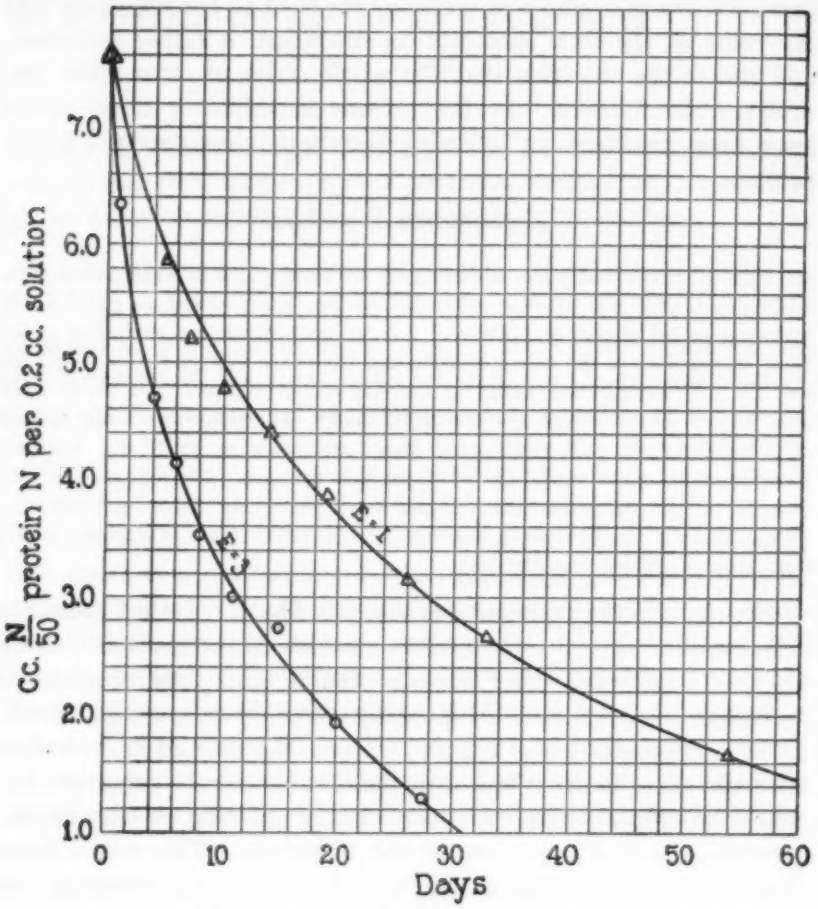


FIG. 1. Rate of digestion of casein solutions at 0° by 1 and by 3 relative trypsin concentrations.

that the square root relation does not hold until the products of the reaction are present in excess. The discrepancy at the end is due to the decrease in the substrate concentration which, in the derivation of Schütz's rule, is assumed constant. It will be noted, however, that the relation holds over a much wider range than could be ex-

pected on the basis of constant substrate, since the equation holds fairly well over a range in which the substrate concentration decreases from 5 to 2. This result is due to the fact that the rate of hydrolysis is not proportional to the total substrate concentration[4] but nearly independent of it when the casein is over 1 per cent. The assumption used to derive Schütz's rule, that the rate of digestion is

TABLE I.

Time Required to Hydrolyze Varying Amounts of Casein with Varying Enzyme.

Protein hydrolyzed per 0.20 cc. as cc. N/50 N.	E = trypsin = 3.		E = trypsin = 1.
	Time.	ET.	Time in days = ET.
	days		
1.60	1.5	4.5	4.8
2.60	3.5	10.5	12
3.60	6.5	19.5	19
4.60	11.5	34.5	28
5.60	19	57	46

Amount hydrolyzed at different times.

ΔT	Protein hydrolyzed.		Ratio: $\frac{\text{Protein hydrolyzed by 3 trypsin}}{\text{Protein hydrolyzed by 1 trypsin}}$
	Trypsin = 3.	Trypsin = 1.	
days			
2	2.0	0.8	2.5
4	2.85	1.4	2.0
10	4.40	2.6	1.7
20	5.6	3.85	1.45
30	6.6	4.80	1.4

independent of the substrate concentration, is therefore experimentally correct over a rather wide range.

Arrhenius' equation, on the other hand, assumes that the rate is directly proportional to the substrate concentration. This is not experimentally the case, so that Arrhenius' correction is too large and the constants for his equation shown in Table II rise toward the end of the reaction. The experimental curve is therefore between the two theoretical curves, since the rate of digestion is not quite independent of the substrate concentration nor is it directly proportional to it.

[4] Northrop, J. H., *J. Gen. Physiol.*, 1923–24, vi, 417.

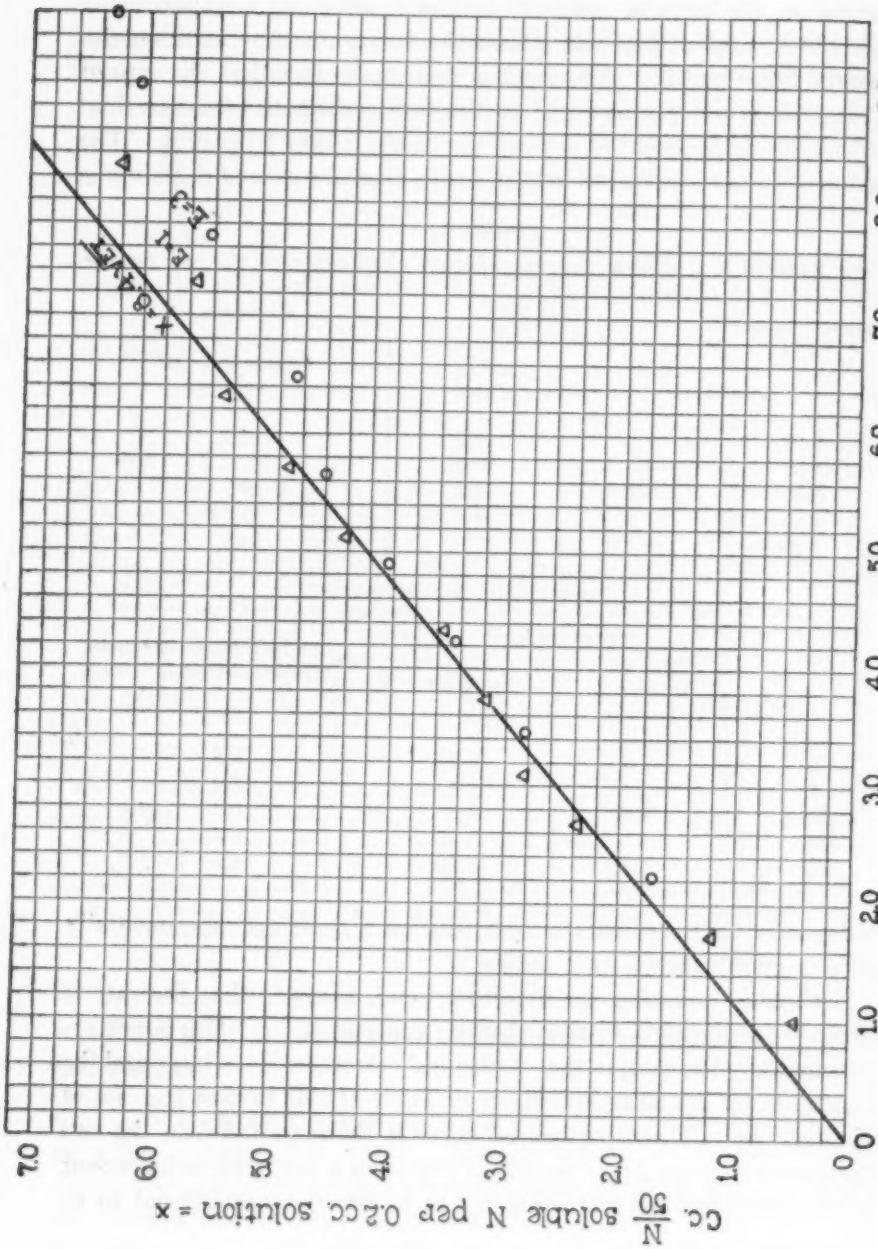


FIG. 2. Rate of digestion of casein solution plotted against the square root of the enzyme concentration × the time in days.

Neither equation expresses accurately the effect of the substrate concentration on the rate of hydrolysis. Schütz's rule is better with high concentrations of substrate and Arrhenius' is better with low concentrations. A similar result was obtained by Dernby.[5]

TABLE II.

Comparison of Arrhenius' Equation (K_A) and Schütz's Rule (K_S) Applied to the Digestion of Casein by Trypsin.

$E = 3$				$E = 1$			
Time.	$A - x$	$K_S = \frac{x}{\sqrt{ET}}$	$K_A = \frac{A \ln \frac{A}{A-x} - x}{ET} \times 10$	Time.	$A - x$	$K_S = \frac{x}{\sqrt{ET}}$	$K_A = \frac{A \ln \frac{A}{A-x} - x}{ET} \times 10$
days				*days*			
0	7.60			0	7.60		
1	6.38	0.74	0.33	3	6.40	0.70	0.3
4	4.68	0.85	0.62	5	5.86	0.78	0.5
6	4.15	0.84	0.63	14	4.40	0.85	0.67
8	3.52	0.82	0.74	19	3.85	0.85	0.74
11	3.00	0.80	0.74	26	3.17	0.87	0.85
20	1.95	0.73	0.78	33	2.65	0.87	0.82
27	1.27	0.70	0.89	40	2.08	0.87	1.00
				54	1.65	0.80	1.00

SUMMARY.

The rate of digestion of concentrated casein solutions by low concentrations of trypsin at 0° has been followed. Under these conditions the enzyme is inhibited by the product of the reaction and under certain conditions this effect should lead to Schütz's rule, *i.e.* the amount of hydrolysis should be proportional to the square root of the product of the time into the enzyme concentration. This is the result obtained. Both Schütz's rule and Arrhenius' equation fail to hold accurately owing to the incorrect relation assumed to hold between the rate of hydrolysis and the substrate concentration.

[5] Dernby, K. G., *Z. physiol. Chem.*, 1914, lxxxix, 425.

FIG. 2. Rate of digestion of casein solution plotted against the square root of the enzyme concentration × the time in days.

PHOTOCHEMISTRY OF VISUAL PURPLE.

III. The Relation Between the Intensity of Light and the Rate of Bleaching of Visual Purple.

By SELIG HECHT.*

(From the Department of Physical Chemistry in the Laboratories of Physiology, Harvard Medical School, Boston.)

(Received for publication, May 23, 1924.)

I.

1. In the reception of light by the eye a common variable is the intensity of the illumination. Indeed, in order to analyse phenomena like dark adaptation and intensity discrimination it has been necessary to assume (Hecht, 1921–22, *c;* 1923–24, *d*) certain relations between the intensity of light and its action on the photosensitive material in the eye.

The idea that visual purple is the sensitive substance concerned with rod vision has had support in an interesting array of quantitative evidence (Koenig, 1894; Trendelenburg, 1904; Hecht and Williams, 1922–23). More recently (Weigert, 1921, *a;* 1922, *b;* Hecht and Williams, 1922–23) it has even seemed possible that visual purple may be concerned with cone vision as well. It is therefore pertinent to a study of the visual process to determine experimentally the influence of the light intensity on the bleaching of visual purple.

2. The chemical change produced by light is proportional to the amount of energy absorbed by the sensitive material (von Grotthuss, 1819; Lasareff, 1907; Luther and Weigert, 1905; Plotnikow, 1907; Weigert, 1922, *c*). The form which this relation takes with visual purple is a simple one. Let a be the initial concentration of visual purple, and let it be exposed for the time t to light of intensity I_0 in

* National Research Fellow.

such a way that it absorbs an amount I_a, and as a result the quantity x is bleached. Then (Plotnikow, 1920)

$$\frac{d(a-x)}{dt} = k I_a \tag{1}$$

where k is a constant. The relation between the incident light I_0 and the absorbed light I_a is according to Beer's law

$$I_a = I_0 (1 - e^{-m(a-x)}) \tag{2}$$

m being the absorption coefficient. Since the concentration of visual purple in the solution used in these experiments is quite small (*cf.* Garten, 1907[1]) equation (2) may be simplified. The exponential $e^{-m(a-x)}$ may be expanded into the series

$$e^{-m(a-x)} = 1 - m(a-x) + \frac{m^2(a-x)^2}{1 \cdot 2} \ldots\ldots \tag{3}$$

Since $(a-x)$ is small, it is safe to neglect all the terms in equation (3) which involve powers higher than unity. The series then reduces to $1-m(a-x)$ and equation (2) becomes

$$I_a = I_0 m (a-x). \tag{4}$$

This value for I_a may now be substituted in equation (1). Remembering that the incident intensity I_0 remains constant in a given experiment, we may write

$$K = k m I_0. \tag{5}$$

Equation (1) then becomes

$$\frac{d(a-x)}{dt} = K(a-x) \tag{6}$$

which when integrated gives

$$K = \frac{1}{t} \log \frac{a}{a-x} \tag{7}$$

the familiar exponential law corresponding to the kinetics of a reaction of the first order.

3. These deductions involve two steps which can be verified ex-

[1] Garten (1907), p. 168.

perimentally. One is that the bleaching reaction follows a monomolecular course (equation (7)). In the first paper of this series, and indirectly in the second as well (Hecht, 1920–21, *a*, *b*) evidence was presented which shows that this is true within the limits of error of the colorimetric method. This, as is clear from the above equations, means that the rate of bleaching at any moment is proportional to the amount of light absorbed, which in turn is a function of the concentration (equation (4)).

Another step in the above reasoning is the assumption that the velocity constant K of the bleaching reaction is directly proportional to the intensity of illumination (equation (5)). It is the purpose of the present work to test this experimentally.

II.

1. The experiments were done by two different methods. Three groups of experiments were made with a colorimetric method and a fourth group with a spectrophotometric method. The colorimetric method has already been described in the first paper of this series. It consists essentially in estimating the concentration of visual purple exposed to light by matching its color in a capillary tube against standards made up of a series of relative concentrations of bleached and unbleached visual purple contained in similar capillary tubes. A set of experiments, made in a single day, consists of measurements of the course of bleaching of visual purple at a number of different intensities. Group I consists of four such sets of experiments; Group II of one set, and Group III of three sets.

2. Group IV, composed of five sets of experiments, was differently performed. A cell was constructed which allows spectrophotometric readings to be made with 1 cc. of solution. The cell serves as well for the vessel in which visual purple is exposed to light, so that no transfer of material is necessary. Exactly 1 cc. of visual purple solution is pipetted into the cell and its absorption measured at 520 $m\mu$, ten settings of the nicols being made for the purpose. The material is then exposed to a given intensity for a chosen time, during which the contents of the cell are stirred evenly and automatically by a motor-driven spirally twisted stirring rod. The cell is then removed and measured, after which it is replaced in front

of the bleaching light, exposed for a further length of time, and again measured. By means of preliminary experiments the exposures were adjusted to give the same amount of bleaching for the various intensities.

The absorption readings are made in terms of photometric density, D, which may be defined in terms of Beer's law (equation (2)) as

$$D = m(a - x) = \log \frac{I_0}{I_t} \quad (8)$$

where I_t is the transmitted light. From this it is apparent that the concentration of a substance varies directly as its photometric density. The readings give the relative concentrations of visual purple present after specific exposures to light, provided a correction is made for the photometric density of the material remaining after the visual purple has been completely bleached. A set of experiments is made in 1 day with the same preparation of visual purple.

The absorption measurements are made at 520 $m\mu$ with a Hilger constant deviation spectrophotometer of the Nutting type. By placing a green filter (Wratten No. 63) between the source of light and the cell, only the part of the spectrum around 520 $m\mu$ falls on the visual purple solution. The wave length 520 $m\mu$ was chosen for measurement because it lies between the maximum absorption of visual purple and the maximum visibility of the spectrum for the fovea and because the absorption represents mostly visual purple and comparatively little of the additional yellow materials which are extracted with it from the retina by the bile salts or are formed from it as an intermediary product of bleaching (Kühne, 1879, Koenig, 1894; Köttgen and Abelsdorff, 1896; Garten, 1907). The source of light for the measurements is a 15 watt concentrated filament lamp, which is kept on only during the actual setting of the nicols.

All the work, and all the readings are made in a dark room, and the spectrophotometer is so arranged that the cell is shielded from every possible stray illumination. As a result of these precautions it is possible to make a large number of readings without perceptibly changing the absorption of the solution. In an experiment made to test this point, forty successive settings of the nicols were recorded

in the usual way in groups of ten. The four averages of the photometric density are 0.376, 0.375, 0.375, and 0.379, showing that the light used in measurement is not a source of error.

3. The bleaching intensities, ranging between 10 and 1,000 meter candles are obtained by varying the distance of the visual purple from a concentrated filament lamp. In Groups I and II the source was a 250 watt lamp; in Group III it was a 500 watt lamp; and in Group IV it was a 400 watt lamp. The intensities were calculated by the inverse square law.

4. The experiments were made at four different times, each group representing a separate period of experimentation. The solutions of visual purple were prepared so that the experiments within the individual groups involve similar concentrations. The absolute values of the concentrations are unknown at present because visual purple has never been isolated as a pure substance. The concentrations in Groups I and II represent four frog retinas to 1 cc. of solution; in Group III about three retinas to 1 cc. of solution; and in Group IV about one retina to 1 cc. of solution. The colorimetric method uses about 0.1 cc. for each experiment, whereas the spectrophotometric method requires about 1 cc., but of a diluter solution.

III.

1. The results are presented in Table I. Each figure in Group I is the average value of K for four experiments; in Group II for one experiment; in Group III for three experiments; and in Group IV for five experiments. It is apparent from the table that the velocity constant of the bleaching reaction varies directly with the intensity of the illumination.

To show the exact nature of this relation the data are plotted in Fig. 1, from which it is clear that the velocity constant is a linear function of the intensity. This is true not only for the experiments as a whole, but for the individual groups as well. The separate groups are identifiable in Fig. 1, and examination of their respective points will show that each group is best represented by a straight line.

2. A word of explanation of the mode of presentation of the data is necessary. The values in Table I of the velocity constants of

Group I are given as computed from the original observations by means of equation (7). Since, however, the amount of solution used as well as the surface exposed to the light varies slightly from group to group, the velocity constants calculated from the different series of experiments are not identical, though nearly so. In order to present them here, the velocity constants of the other three groups are multiplied by a factor, as given in Table I for each group. The factors are arbitrarily chosen as follows. The data from each group

TABLE I.

Relation between the Velocity Constant K and the Intensity.

Intensity.	$K = \frac{1}{t} \log \frac{a}{a-x}$			
	I × 1.00	II × 0.704	III × 0.870	IV × 0.895
m. c.				
12.3		0.00774		
14.0			0.00454	
27.5	0.0144	0.0148		
52.5	0.0277	0.0267		
60.0				0.0281
100.0			0.0395	0.0469
110.0	0.0463	0.0566		
200.			0.0732	
250.	0.120	0.102		
300.			0.136	0.127
500.			0.217	
510.	0.229	0.237		
600.				0.258
690.			0.316	
1,000.	0.439		0.447	0.452

are plotted and a straight line drawn through them. The factor then is the fraction by which the tangent of the line must be multiplied in order that the line will coincide with that drawn for Group I.

This procedure does not in the least influence the nature of the results. The numerical values of the velocity constants have no theoretical meaning by themselves in the present state of our knowledge of visual purple; it is only their relative values which are of significance. And the present experiments leave little doubt that

the velocity of bleaching of visual purple under comparable conditions of concentration, volume, and surface exposed is directly proportional to the intensity of the incident light.

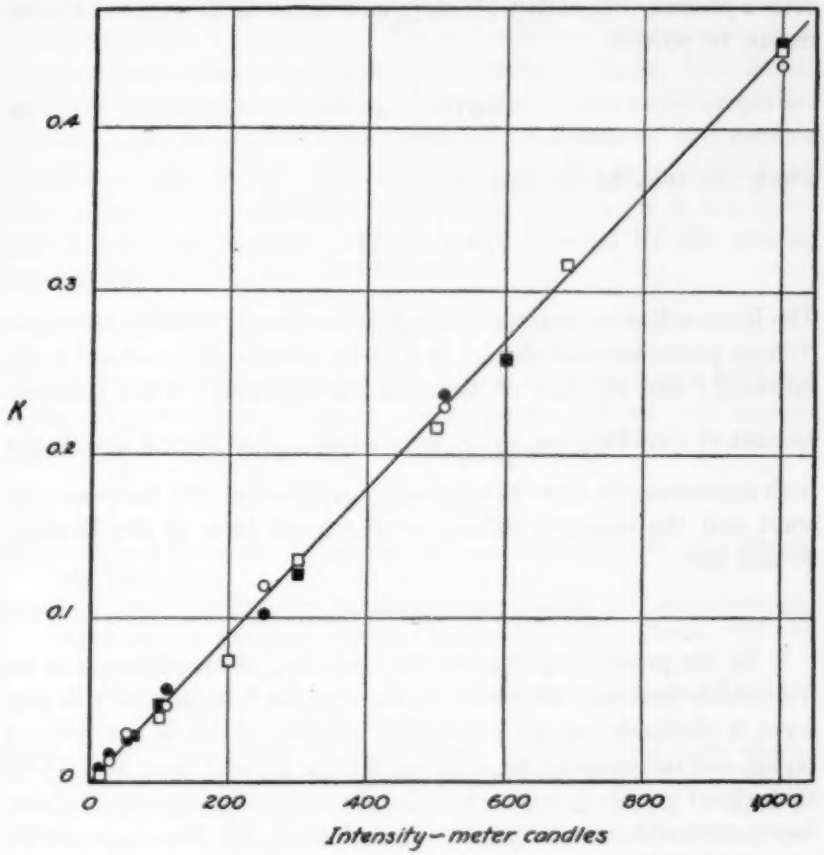


Fig. 1. Relation between the velocity constant K of the bleaching reaction and the intensity of the light.

IV.

1. The results of these experiments are a confirmation of the Bunsen-Roscoe law. The validity of this law has been questioned by those who have tested it on complicated reactions like the photographic process (Schwarzschild, 1899). However, as originally con-

ceived by Bunsen and Roscoe (1862) it probably applies only to simple irreversible photochemical reactions. The bleaching of visual purple under the conditions of these experiments is an example of such a process. Equation (7) may, as a result of the present experiments, be written

$$k\,m\,I = \frac{1}{t}\log\frac{a}{a-x} \tag{9}$$

which can then be changed to

$$I\,t = \frac{1}{k\,m}\log\frac{a}{a-x}. \tag{10}$$

The Bunsen-Roscoe reciprocity law requires that in order to produce a definite photochemical change in a given system the product of the intensity I and the time of its action t be constant. For a constant amount of bleaching $\log\frac{a}{a-x}$ is constant. And since k and m are both constants, the right hand member of equation (10) becomes constant and the equation reduces to the usual form of the Bunsen-Roscoe law

$$I\,t = \text{constant}. \tag{11}$$

2. In the present experiments the bleaching of visual purple is an irreversible process. However, in the eye, the bleaching of this pigment is distinctly not an irreversible process. Even in the isolated retina, and occasionally in solution, Kühne showed years ago (1879) that visual purple is regenerated in the dark. The system is therefore a reversible one. Very likely, as Luther and Plotnikow (1908) suggested, it is a pseudoreversible reaction in which one of the substances necessary for regeneration has to be freshly supplied. However, since with an excess present of the accessory substance necessary for the dark reaction, the behavior of the pseudoreversible reaction simulates a completely reversible reaction, it is advisable to be non-committal and call the visual purple system in the retina simply a reversible one, the chemical conditions for regeneration being at present unknown.

The effect of light on such a reversible system is less than on the same system in an irreversible state, and the difference depends on the speed of the dark reaction. Certain evidence (Hecht, 1921–22, *c*) leads us to suppose that the kinetics of the "dark" reaction are bimolecular, perhaps trimolecular. The quantitative results of such a situation have already been gone into elsewhere (Hecht, 1923–24, *d*) and need not be repeated here. They indicate that some properties of vision can be described quantitatively in terms of a reversible reaction of this nature. One assumption in this system has been that the velocity constant of the bleaching reaction *per se* is a linear function of the intensity. This is now supported by the present experiments.

SUMMARY.

It is shown that the velocity of bleaching of visual purple by light, under comparable conditions of concentration, volume, and surface exposed, is directly proportional to the intensity.

BIBLIOGRAPHY.

Bunsen, R., and Roscoe, H., Photochemische Untersuchungen. IV, *Ann. Physik*, 1862, cxvii, 529.

Garten, S., Die Veränderungen der Netzhaut durch Licht, in Graefe-Saemisch, Handbuch der Gesamten Augenheilkunde, 2nd edition, Berlin, 1907, iii, Chapter XII, suppl.

von Grotthuss, T., Ueber die chemische Wirksamkeit des Lichts und der Electricität, *Ann. Physik*, 1819, lxi, 50.

Hecht, S., (*a*) Photochemistry of visual purple. I. The kinetics of the decomposition of visual purple by light, *J. Gen. Physiol.*, 1920–21, iii, 1; (*b*) II. The effect of temperature on the bleaching of visual purple by light, *J. Gen. Physiol.*, iii, 285; (*c*) The nature of foveal dark adaptation, *J. Gen. Physiol.*, 1921–22, iv, 113; (*d*) Intensity discrimination and the stationary state, *J. Gen. Physiol.*, 1923–24, vi, 355.

Hecht, S., and Williams, R. E., The visibility of monochromatic radiation and the absorption spectrum of visual purple, *J. Gen. Physiol.*, 1922–23, v, 1.

Koenig, A., Ueber den menschlichen Sehpurpur und seine Bedeutung für das Sehen, *Sitzungsber. k. Akad. Wissensch.*, 1894, 577; reprinted in Koenig, A., Gesammelte Abhandlungen, Leipsic, 1903.

Kühne, W., Chemische Vorgänge in der Netzhaut, in Hermann, L., Handbuch der Physiologie, Leipsic, 1879, iii, pt. 1, 235.

Lasareff, P., Über das Ausbleichen von Farbstoffen im sichtbaren Spektrum, *Ann. Physik*, 1907, xxiv, 661.

Luther, R., and Plotnikow, J., Über scheinbar umkehrbare photochemische Vorgänge und photochemische Übertragungskatalyse, *Z. physik. Chem.*, 1908, lxi, 513.

Luther, R., and Weigert, F., Über umkehrbare photochemische Reaktionen in homogenen System. Anthracen und Dianthracen. I, *Z. physik. Chem.*, 1905, li, 297.

Plotnikow, J., Die photochemische Oxydation von Jodwasserstoff, *Z. physik. Chem.*, 1907, lviii, 214; Allgemeine Photochemie, Berlin and Leipsic, 1920.

Schwarzschild, K., Ueber Abweichungen von Reciprocitätsgesetz für Bromsilbergelatine, *Photograph. Correspondenz*, 1899, xxxvi, 109.

Trendelenburg, W., Quantitative Untersuchungen über die Bleichung des Sehpurpurs in monochromatischem Licht, *Z. Psychol. u. Physiol. Sinnesorg.*, 1904, xxxvii, 1.

Weigert, F., (*a*) Ein photochemisches Modell der Retina, *Arch. ges. Physiol.*, 1921, cxc, 177; (*b*) Zur physikalischen Chemie des Farbensehens, *Z. physik. Chem.*, 1922, c, 537; (*c*) Zur Theorie der Strahlungsumformungen. I. Der allgemeine Mechanismus photochemischer Prozesse, *Z. physik. Chem.*, 1922, cii, 416.

THE ELECTRIC CONDUCTIVITY OF DISPERSE SYSTEMS.

By HUGO FRICKE.

(From the Department of Biophysics, Cleveland Clinic Foundation, Cleveland.)

(Received for publication, May 23, 1924.)

In a paper now in press,[1] I have presented a theory for the conductivity of a suspension of homogeneous spheroids[2] in which the following formula was derived.

$$\frac{k-k_1}{k-k_2}\left(1-\frac{k_2}{k_1}\right)=\beta\cdot\frac{\rho}{1-\rho} \tag{1}$$

k, specific conductivity of suspension.
k_1, " " " suspending medium.
k_2, " " " suspended medium.
ρ, volume concentration " " "

$$\beta=\frac{1}{3}\left[\frac{2}{1+\left(\frac{k_2}{k_1}-1\right)\int_0^\infty\frac{d\lambda}{\left(1+\frac{b^2}{a^2}\lambda\right)^{\frac{1}{2}}\left(1+\lambda\right)^2}}+\right.$$

$$\left.\frac{1}{1+\left(\frac{k_2}{k_1}-1\right)\left(1-\int_0^\infty\frac{d\lambda}{\left(1+\frac{b^2}{a^2}\lambda\right)^{\frac{1}{2}}\left(1+\lambda\right)^2}\right)}\right]\left(\frac{k_2}{k_1}-1\right)$$

a, b, axes of spheroid.

For the case of the sphere $\left(\frac{a}{b}=1\right)$, formula (1) reads[1]

$$\frac{\frac{k}{k_1}-1}{\frac{k}{k_1}+2}=\rho\,\frac{\frac{k_2}{k_1}-1}{\frac{k_2}{k_1}+2} \tag{2}$$

[1] Fricke, H., A mathematical treatment of the electric conductivity and capacity of disperse systems, *Phys. Rev.*, 1924 (in press).

[2] The theory for the simple case of a diluted suspension of spheres was given in a recent paper. Fricke, H., *J. Gen. Physiol.*, 1923–24, vi, 375.

For a diluted solution $\frac{k}{k_1}$ is approximately equal to 1; introducing this value of $\frac{k}{k_1}$, equation (2) reduces to

$$\frac{k}{k_1} = 1 + 3\rho \frac{\frac{k_2}{k_1} - 1}{\frac{k_2}{k_1} + 2} \tag{3}$$

This is the equation, the derivation of which was given in a previous paper.[2]

β is given in graphical form in Fig. 1.

It is of interest to note that according to equation (1) and Fig. 1, for a constant volume concentration of the suspended medium the conductivity of a suspension is independent of the size of the suspended particles and also nearly independent of the form of the particles when the difference between the conductivities of the suspended and the suspending media is not very large. This is especially true for suspensions of prolated spheroids which are less conductive than the suspending medium.

Investigations in which formula (1) is being applied to different problems of practical and theoretical interest are at present being made in this laboratory. In the first of these studies which will be published shortly, the case of cream will be considered. In the present paper the formula will be applied to the case of blood.

The most exact investigations of the conductivity of blood in terms of the volume concentration of the red corpuscles have been made by Stewart[3] and Fraenckel.[4] Stewart has determined the volume concentration by two independent methods, a colorimetric method and the Hoppe-Seyler chemical method. Fraenckel has used Bleibtreu's chemical method. The agreement between the results secured by these investigations is very close. However, as has been stated also by Fraenckel, one would expect that Stewart's methods would give the most exact results and therefore we shall here consider only his measurements. In Table I is given a series of measurements

[3] Stewart, G. N., *J. Physiol.*, 1899, xxiv, 356.

[4] Fraenckel, P., *Z. klin. Med.*, 1904, lii, 470.

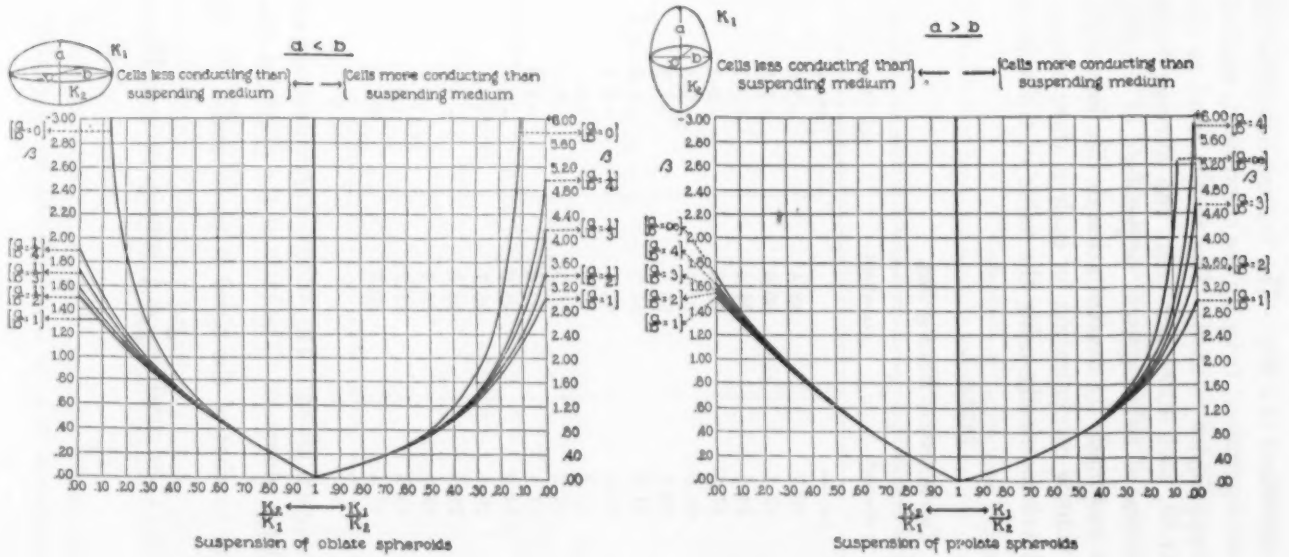


FIG. 1. Graphical representation of β.

Conductivity of suspension of homogeneous spheroids.

$$\frac{K - K_1}{K - K_2}\left(1 - \frac{K_2}{K_1}\right) = \beta \cdot \frac{\rho}{1 - \rho}$$

K, specific conductivity of suspension; K_1, specific conductivity of suspending medium; K_2, specific conductivity of spheroid; ρ, volume concentration of suspension; β, plotted.

made by Stewart[5] for the blood of a dog. The volume concentration of the normal blood was determined twice by the colorimetric method and twice by the Hoppe-Seyler method. The results were as follows: 41.16, 40.72, 41.52, and 40.99 per cent, the average being 40.98 per cent. A series of red corpuscle suspensions of varying volume concentration was made by concentration or dilution of the normal blood. The volume concentration for each of these suspensions was determined volumetrically using the value of the volume

TABLE I.

Conductivity of Blood of Dog.

$\frac{k_1}{k}$	ρ observed.	ρ calculated.	Δ
	per cent		*per cent*
15.62	90.7	88.4	+2.6
9.08	82.1	80.9	+1.5
6.56	74.5	74.4	+0.1
5.06	67.8	68.0	−0.3
4.14	61.6	62.1	−0.8
3.51	56.1	56.8	−1.2
3.063	51.0	51.9	−1.8
2.726	46.4	47.4	−2.2
2.436	42.2	42.9	−1.6
2.348	41.0	41.3	−0.7
2.225	38.4	39.0	−1.6
1.903	31.9	32.0	−0.3
1.697	26.4	26.7	−1.1
1.539	21.8	22.0	−0.9
1.428	18.1	18.3	−1.1
1.342	15.3	15.2	+0.7
1.232	11.4	10.8	+5.5

concentration of the original blood. The volume concentrations are given in Table I under ρ observed. The ratio of the observed conductivity of the serum to that of the blood is given under $\frac{k_1}{k}$. In order to employ formula (1) we consider $\frac{a}{b} = \frac{1}{4}$; consequently in Fig. 1, $\beta = -1.91$.

[5] Stewart,[3] p. 369.

Furthermore, $k_2 = 0$. The formula therefore reads

$$1 - \frac{k_1}{k} = -1.91 \cdot \frac{\rho}{1-\rho}$$

The values of the volume concentration ρ calculated from this formula using the observed values of $\frac{k_1}{k}$ are given under ρ calculated.

TABLE II.

Conductivity of Suspensions of Sand.

$\frac{k_1}{k}$ observed.	ρ observed.	β
	per cent	
2.488	40.0	2.22
2.220	36.4	2.14
2.075	33.3	2.15
1.952	30.8	2.14
1.866	28.6	2.17
1.790	26.7	2.17
1.725	25.0	2.18
1.6835	23.5	2.22

Average value of β, 2.17.

TABLE III.

Conductivity of Suspensions of Sand.

$\frac{k_1}{k}$ observed.	ρ observed.	β
	per cent	
1.190	10	1.710
1.426	20	1.705
1.734	30	1.712
2.156	40	1.732
2.715	50	1.715
3.613	60	1.74

Average value of β, 1.72.

The percentile differences between ρ observed and ρ calculated are given under Δ. The deviations throughout are within the limits of experimental errors.

Before concluding, it may perhaps be of interest to apply our formula to some measurements of sand suspensions which have been

made by Oker-Blom[6] and which often are cited in connection with investigations of the conductivity of blood. Two different kinds of sand were used in these investigations. The suspensions were prepared by adding the sand to a hot salt solution containing 3 per cent gelatin and cooling this mixture to gelatination under continuous rotation. No information is given concerning the form of the sand particles except for the statement that the particles of the sand which were used in the second series of experiments (Table III) were more nearly spherical than those which were used in the first series (Table II). Our theory is therefore best applied by using Oker-Blom's observed values of $\frac{k_1}{k}$ and ρ and calculating the value of β by means of the formula $1 - \frac{k_1}{k} = -\beta \cdot \frac{\rho}{1-\rho}$ obtained from formula (1) by considering $k_2 = 0$. Our calculated results are given in Tables II and III, the sand used in the measurements in the two tables corresponding to the two kinds of sand used by Oker-Blom. The calculated values for β are constant within experimental errors. The deviation of the value of β from 1.50 is a measure of the deviation of the sand particles from the spherical form.

[6] Oker-Blom, M., *Arch. ges. Physiol.*, 1900, lxxix, 510.

THE SOLUBILITY OF TYROSINE IN ACID AND IN ALKALI.

By DAVID I. HITCHCOCK.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, May 6, 1924.)

I.

INTRODUCTION.

Many amphoteric electrolytes form soluble salts with acid or alkali, while the undissociated or isoelectric substances themselves are often only slightly soluble in water. It should, therefore, be possible to calculate the solubility of such a substance from its ionization constants as an acid and as a base and from the hydrogen ion concentration of the saturated solution. The theory underlying this dependence of the solubility on the hydrogen ion concentration has been outlined by Michaelis,[1] who stated, however, that the only experimental investigations of the question have consisted in determinations of the pH at which the solubility was a minimum. A few measurements were made by Beveridge[2] of the solubility of anthranilic acid in hydrochloric acid, but the work was hardly extended far enough to determine whether the increased solubility could be theoretically explained. Cohn and Hendry[3] studied the effect of hydrogen ion concentration on the solubility of casein in very dilute solutions of sodium hydroxide, but this case was complicated by the fact that the constitution, molecular weight, and basicity of the ampholyte were unknown. Accordingly it seemed advisable to measure the solubility in acid and in alkali of a comparatively simple ampholyte of known constitution, with the object of testing the theoretical relation between the solubility and hydrogen

[1] Michaelis, L., Die Wasserstoffionenkonzentration, Berlin, 2nd edition, 1922, 73, 58.

[2] Beveridge, H. H., *Proc. Roy. Soc. Edinburgh*, 1908–09, xxix, 648.

[3] Cohn, E. J., and Hendry, J. L., *J. Gen. Physiol.*, 1922–23, v, 521.

ion concentration. The substance used in this work was *l*-tyrosine, or para-hydroxyphenyl-α-amino-propionic acid, $HO \cdot C_6H_4 \cdot CH_2 \cdot CHNH_2 \cdot COOH$.

II.

EXPERIMENTAL PROCEDURE.

The tryosine was prepared from commercial casein hydrolyzed by boiling with hydrochloric acid, following the procedure of Abderhalden[4] (who, however, used silk as the raw material). The product was decolorized with animal charcoal, recrystallized three times from hot water, and dried with alcohol and ether. Its nitrogen content, determined by the Kjeldahl method, was found to be 7.73 per cent; calculated, 7.733.

Saturated solutions of tyrosine in HCl or NaOH of various concentrations were obtained by placing 100 cc. of the solvent in contact with an excess of solid tyrosine in 150 cc. Pyrex flasks closed tightly by rubber stoppers, and rotating the flasks, end over end, for 1 or 2 days in a water bath at 25°C. ± 0.05°. Preliminary measurements of the solubility gave identical values after 1 and after 2 days in the thermostat. In the case of pure water it was found that equilibrium was reached more slowly; concordant values were obtained only after 3 days agitation of tyrosine with supersaturated and undersaturated solutions. The solutions were filtered with suction through hardened filter paper fastened by a rubber band over the end of a glass tube about 6 mm. in diameter, which was connected by rubber tubing to a pipette. This made it possible to conduct the filtration without removing the flasks from the thermostat.

The concentration of tyrosine in the saturated solutions was obtained by Kjeldahl determinations of nitrogen, using 100 cc. Kjeldahl flasks with 2 cc. of concentrated H_2SO_4, 0.5 cc. of 5 per cent $CuSO_4$, 1 gm. of K_2SO_4, and a few pieces of broken alundum to prevent bumping. For the lower concentrations of tyrosine 25 cc. samples were used; for the higher concentrations, suitable aliquot parts. After the addition of 50 cc. of water and 6 cc. of 1:1 sodium

[4] Abderhalden, E., *Z. physiol. Chem.*, 1912, lxxvii, 75.

hydroxide solution, the ammonia was distilled into 25 cc. of N/50 HCl, and determined by titration with N/50 NaOH, using methyl red as indicator. The blanks obtained with the reagents varied from 0.2 to 0.3 cc. N/50. Duplicate analyses generally agreed to within 0.1 cc. N/50. Each solubility determination represents the mean of two analyses; the figure for tyrosine and water is the mean of those obtained from eight separate solutions.

The hydrogen ion concentrations were determined by electromotive force measurements with Clark electrodes in an air thermostat at 25°C. They are based on the value 1.035 for the pH of 0.1000 M HCl, and on the assumption that contact potential differences were eliminated by the use of saturated KCl. The concentrations of hydroxyl ions in the alkaline solutions were calculated from the pH measurements, using the value $k_w = 1 \times 10^{-14}$ for the ion product of water at 25°.

The concentrations of HCl and NaOH given are those of the original solutions with which the tyrosine was shaken. It was found by direct titration, using methyl red, that the solution of the tyrosine did not appreciably change these values.

The results are given in Tables I and II.

III.

DISCUSSION OF RESULTS.

The results obtained with hydrochloric acid are plotted in Fig. 1. It is evident that the solubility of tyrosine is a linear function of the initial concentration of hydrochloric acid, and also of the final concentration of hydrogen ion. That this is predicted by the theory may be shown as follows. Representing tyrosine by TOH, in acid solutions it will ionize as a base:

$$TOH \rightleftarrows T^+ + OH^-$$

Applying the law of mass action,

$$\frac{[T^+]\,[OH^-]}{[TOH]} = k_b$$

Since $[H^+]\,[OH^-] = k_w$, the ion product of water, letting $K = \frac{k_b}{k_w}$ $s_0 = [TOH]$, $h = [H^+]$, and $t = [T^+]$, it follows that

$$K = \frac{t}{s_0 h} \tag{1}$$

TABLE I.

Solubility of Tyrosine in Hydrochloric Acid.

Concentration of HCl, mols per liter × 10^3.	Concentration of tyrosine, mols per liter × 10^3.	pH	Concentration of hydrogen ion, mols per liter × 10^5.
0	2.62	5.1 to 5.5	0.01
1.00	3.09	3.19	0.65
2.00	3.25	2.857	1.39
4.99	4.10	2.457	3.49
9.99	5.39	2.160	6.92
20.00	8.43	1.861	13.8
30.00	10.8	1.675	21.1
40.00	13.8	1.560	27.6
50.00	16.5	1.450	35.5

TABLE II.

Solubility of Tyrosine in Sodium Hydroxide.

Concentration of NaOH, mols per liter × 10^3.	Concentration of tyrosine, mols per liter × 10^3.	pH	Concentration of hydroxyl ion, mols per liter × 10^5.
0	2.62	5.1 to 5.5	0.000
0.98	3.54	8.342	0.220
1.95	4.30	8.865	0.731
4.88	7.06	9.249	1.775
9.76	10.7	9.484	3.05
19.5	17.5	9.726	5.32
29.9	24.7	9.841	6.94
39.8	30.4	9.881	7.60
49.8	35.8	9.953	8.98

The total solubility of tyrosine, s, is equal to the concentration of the undissociated molecule plus that of the tyrosine chloride formed, or

$$s = s_0 + \frac{t}{d_s} \tag{2}$$

if d_s = the degree of dissociation of the salt formed. The total concentration of hydrochloric acid, a, is equal to that of the free hydrochloric acid remaining plus that of the tyrosine chloride formed, or

$$a = \frac{h}{d_a} + \frac{t}{d_s} \tag{3}$$

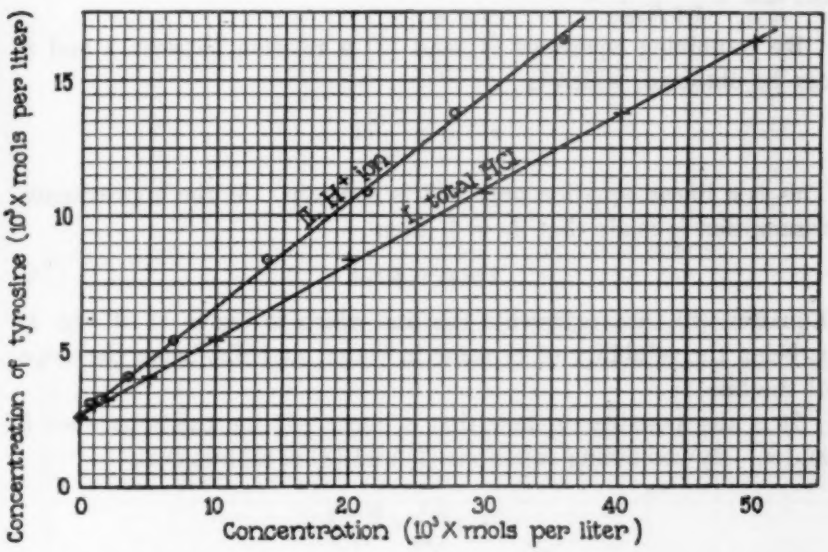


FIG. 1. Effect of the concentration of total HCl and of H^+ ion on the solubility of tyrosine. The curves are linear, as the theory requires for a mono-acid base.

where d_a is the degree of dissociation of the hydrochloric acid. By combining these three equations the following expression is obtained for s in terms of a.

$$s = s_0 + \frac{K s_0}{\frac{d_s}{d_a} + K s_0} a$$

Assuming that $d_s = d_a$ (which should be approximately true in accordance with the isoionic principle and the rule of equal ionization for strong electrolytes of the same type[5]), the relation becomes

[5] Washburn, E. W., Principles of physical chemistry, New York and London, 1st edition, 1915. 317, 295, 220.

$$s = s_0 + \frac{K s_0}{1 + K s_0} a \tag{4}$$

This equation indicates the linear relationship between s and a, in Curve I of Fig 1. Evidently s_0 is, within the experimental error, equal to the solubility of tyrosine in pure water, and the slope of the line is $\frac{K s_0}{1+Ks_0}$.

By combining equations (1) and (2) a relation between s and h can be obtained, which is

$$s = s_0 + \frac{K s_0}{d_s} h$$

This is a linear equation only if d_s is constant. If the approximate assumption is made that $d_s = 1$, then

$$s = s_0 + K s_0 h \tag{5}$$

Equation (5) thus represents the line which is Curve II of Fig. 1, s_0 being the solubility of tyrosine in water, and Ks_0 being the slope of the line.

It is also possible to show that a linear relation exists between h and a. By combining equations (1) and (3) it follows that

$$a = h\left(\frac{1}{d_a} + \frac{K s_0}{d_s}\right)$$

If it is assumed that $d_a = d_s = 1$, then

$$a = h\,(1 + K s_0) \tag{6}$$

Equations (4), (5), and (6) can be tested by using the experimental values of s_0, s, a, and h to calculate K, and hence k_b, which is the product of K and k_w. The results of this calculation are given in Table III, using 1.0×10^{-14} as the value of k_w at 25°. The best value for k_b, 1.57×10^{-12}, is probably more reliable than the value 2.6×10^{-12}, which was found by Kanitz.[6] The older value was obtained by the conductivity method, which involves a doubtful estimation of the conductivity of unhydrolyzed tyrosine chloride. Attempts to obtain this quantity by experiment in this laboratory have been fruitless.

[6] Kanitz, A., *Arch. ges. Physiol.*, 1907, cxviii, 539.

TABLE III.

Basic Ionization Constant of Tyrosine.

Valves for $k_b \times 10^{13}$ as calculated from.			s (observed) $\times 10^3$.	s (calculated) $\times 10^3$.
a and s.	k and s.	k and a.		
			2.62	2.62
(3.4)	(2.8)	(2.1)	3.09	2.89
1.8	1.7	1.7	3.25	3.19
1.6	1.6	1.6	4.10	4.06
1.5	1.5	1.7	5.39	5.47
1.6	1.6	1.7	8.43	8.29
1.4	1.5	1.6	10.8	11.3
1.5	1.6	1.7	13.8	14.0
1.5	1.5	1.6	16.5	17.2
Mean....1.56	1.57	1.66	$k_b = 1.57 \times 10^{-13}$	

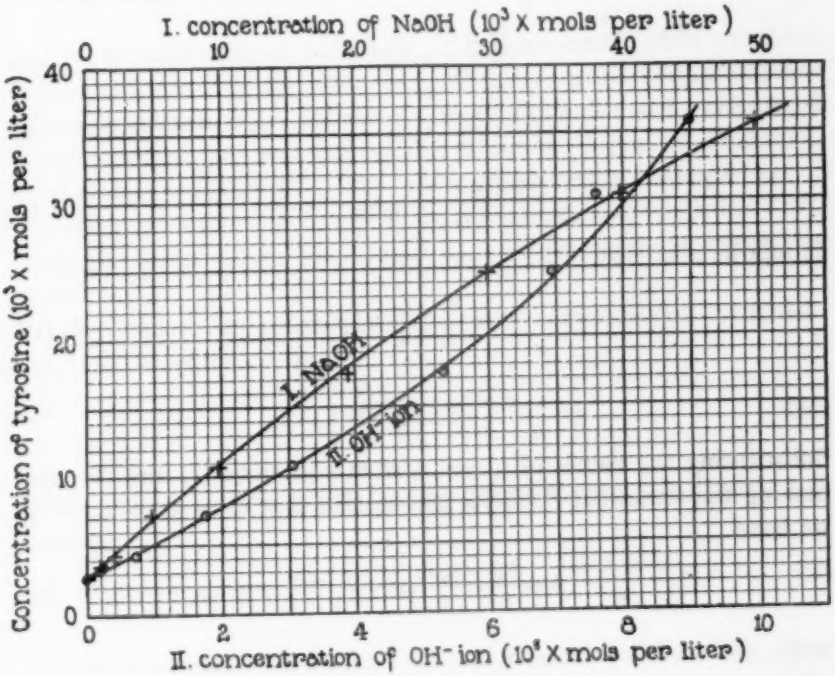


FIG. 2. Effect of the concentration of total NaOH and of OH^- ion on the solubility of tyrosine. The curves are not linear because tyrosine is a dibasic acid.

The constancy of the values for k_b indicates the extent to which the experimental data agree with the theory and the assumptions made. The agreement of the observed values of s with those calculated from equation (5) indicates more directly, perhaps, that the data are satisfactorily explained by the theory.

The results obtained with tyrosine and sodium hydroxide are plotted in Fig. 2. Here the curves are not linear; and the explanation for this is to be found in the fact that tyrosine acts as a dibasic acid, the phenol group as well as the carboxyl group losing hydrogen ions.

Representing the total concentration of sodium hydroxide by b, its degree of ionization by d_b, the ionization of monosodium tyrosinate by d_1, the concentration of its negative ion by t_1, the ionization of disodium tyrosinate by d_2, and the concentration of its negative ion by t_2, the following equations result from the application of the law of mass action to the ionization of tyrosine as a dibasic acid.

$$\frac{t_1 h}{s_0} = k_{a1} \tag{7}$$

$$\frac{t_2 h}{t_1} = k_{a2} \tag{8}$$

$$\frac{t_2 h^2}{s_0} = k_{a1} k_{a2} \tag{9}$$

It follows also that

$$b = \frac{k_w}{h d_b} + \frac{t_1}{d_1} = \frac{2 t_2}{d_2} \tag{10}$$

$$s = s_0 + \frac{t_1}{d_1} + \frac{t_2}{d_2} \tag{11}$$

Substituting the values of t_1 and t_2 from equations (7) and (9), and making the somewhat doubtful assumption that $d_b = d_1 = d_2 = 1$, we have

$$b = \frac{k_w}{h} + \frac{k_{a1} s_0}{h} + \frac{2 k_{a1} k_{a2} s_0}{h^2} \tag{12}$$

and

$$s = s_0 + \frac{k_{a1} s_0}{h} + \frac{k_{a1} k_{a2} s_0}{h^2} \tag{13}$$

By combining equations (12) and (13) it is possible to calculate approximate values for k_1 and k_2. Thus it would follow that

$$b - s = \frac{k_w}{h} + \frac{k_{a1} k_{a2} s_0}{h^2} - s_0$$

and

$$b - 2\,s = \frac{k_w}{h} - 2\,s_0 - \frac{k_{a1}\,s_0}{h}$$

from which

$$k_{a1} = \left(\frac{k_w}{h} + 2\,s - 2\,s_0 - b\right)\frac{h}{s_0} \tag{14}$$

and

$$k_{a1}\,k_{a2} = \left(b + s_0 - s - \frac{k_w}{h}\right)\frac{h^2}{s_0} \tag{15}$$

It is to be noted that equations (7) to (11) are exact, while equations (12) to (15) are only approximate, since they contain the assumption of complete ionization. However, the values of k_{a1} and k_{a2} calculated from equations (14) and (15) exhibit a certain degree of constancy, as is shown in Table IV. The table contains also a comparison of the observed values of s with those calculated from equation (13), using the mean values for k_{a1} and k_{a2} and the observed values for s_0 and h.

The complete expression for the solubility of tyrosine at any hydrogen ion concentration is

$$s = s_0\left(1 + \frac{k_1\,h}{k_w} + \frac{k_{a1}}{h} + \frac{k_{a1}\,k_{a2}}{h^2}\right) \tag{16}$$

If the values already given for the constants are substituted, this becomes

$$s = 0.00262\left(1 + 157\,h + \frac{7.8 \times 10^{-10}}{h} + \frac{6.6 \times 10^{-20}}{h^2}\right) \tag{17}$$

The curve in Fig. 3 represents values of s calculated from this equation, plotted as a function of the pH. The circles represent the values of s experimentally determined.

TABLE IV.

Acidic Ionization Constants of Tyrosine.

$k_{a1} \times 10^{10}$	$k_{a1}k_{a2} \times 10^{20}$	s (observed) $\times 10^3$.	s (calculated) $\times 10^3$.
(14.9)	(47)	3.54	3.08
7.4	(18)	4.30	4.20
8.7	5.1	7.06	6.79
8.1	6.8	10.7	10.5
7.4	6.1	17.5	18.4
7.9	6.1	24.7	25.2
8.0	7.9	30.4	28.2
7.1	7.8	35.8	34.9
Mean....7.8	Mean....6.6	$k_{a1} = 7.8 \times 10^{-10}$ $k_{a2} = 8.5 \times 10^{-11}$	

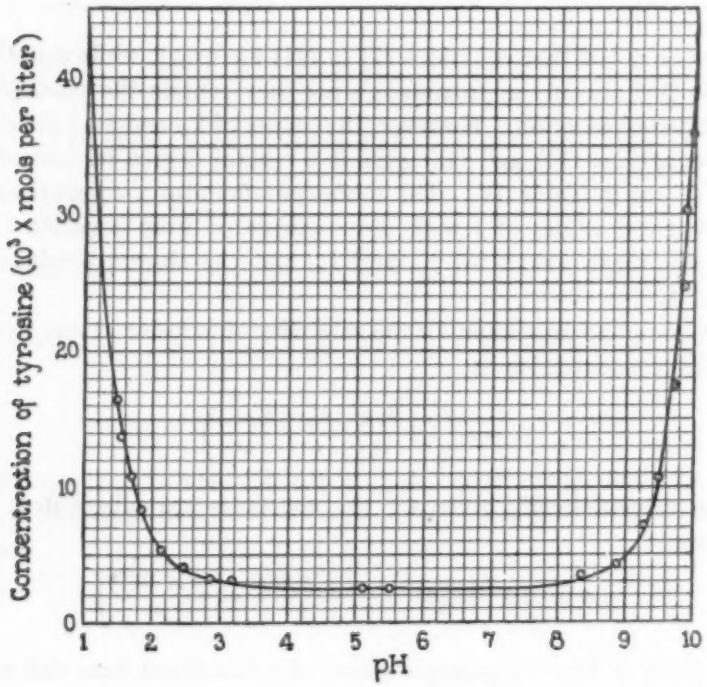


FIG. 3. Effect of pH on solubility of tyrosine. The points represent observed values; the curve represents values calculated from the theory.

In equation (16) the expression in parentheses is the reciprocal of the quantity designated by Michaelis[7] as ρ, the unionized fraction of the ampholyte, which in the notation used here is $\frac{s_0}{s}$. By differentiating his expression with respect to h and placing the derivative equal to zero, an equation can be derived for the hydrogen ion concentration at the isoelectric point, as was done by Michaelis for the case of an ampholyte with only one acidic group. Substituting the values of the constants, this calculation gives for the isoelectric point

$$h^3 = 5 \times 10^{-12} h + 8.4 \times 10^{-22} \qquad (18)$$

From the shape of the curve in Fig. 3, the isoelectric point would appear to lie between pH 5 and 6. If this is the case the last term in equation (18) is negligible, and the value for h becomes 2.23×10^{-6}, corresponding to pH 5.65. The same value is obtained by the equation of Michaelis, which considers from the start only one acid and one basic ionization constant. In such a case as this, however, as Michaelis has pointed out, the exact location of the isoelectric point has little significance, since the ampholyte is isoelectric or almost completely undissociated over a broad zone of pH values.

SUMMARY.

Measurements have been made of the solubility at 25°C. of tyrosine in hydrochloric acid and in sodium hydroxide solutions varying from 0.001 to 0.05 M, and also in distilled water. The pH of the saturated solutions was measured with the hydrogen electrode. The following values for the ionization constants of tyrosine have been obtained from the measurements: $k_b = 1.57 \times 10^{-12}$, $k_{a1} = 7.8 \times 10^{-10}$, $k_{a2} = 8.5 \times 10^{-11}$. The changes in solubility with pH can be satisfactorily explained by the use of these ionization constants.

[7] Michaelis[1], p. 54.

APPLICATION OF THE DIFFUSION HYPOTHESIS TO MEMBRANE POTENTIALS.

By CECIL D. MURRAY.

(From the Department of Physiology, Columbia University, New York.)

(Received for publication, May 27, 1924.)

In a paper on the acid-base equilibrium in two phase systems[1] the following case was suggested as an interesting one from the point of view of the Donnan equilibrium.

Two aqueous solutions, A and B, contain equal concentrations of undissociated lactic acid (HL), but different concentrations of Na-lactate (NaL). The solutions are separated by a layer of amyl alcohol in which there is a sufficient concentration of HL to be in equilibrium with both aqueous solutions, but in which the NaL is practically insoluble. The condition is approximately the one which would be obtained if amyl alcohol containing HL, in excess of the total base present, were shaken up several times alternately with two aqueous solutions containing originally two different concentrations of NaOH. The conditions of this equilibrium and the limitations of the mathematical treatment are fully described in the paper above.

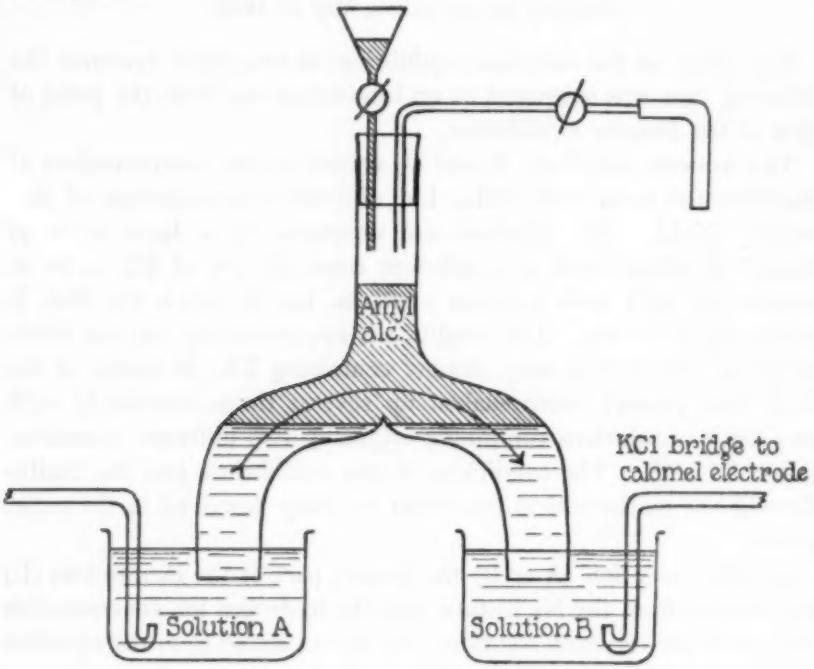
In either solution (A or B) the greater part of the lactate ions (L) are derived from the Na lactate, and the hydrogen ion concentration (except in the very acid solutions) can be calculated from the equation

$$H = \frac{HL}{NaL} \times \frac{K}{\gamma}; \text{ or } pH = \log \frac{NaL}{HL} + pK' \qquad (pK' = 3.65)$$

At equilibrium, $HL_A = HL_B$, therefore $H_A \times L_A = H_B \times L_B$. Hence the solution with the lower concentration of NaL will have a lower concentration of L and a higher concentration of H. The conditions of the Donnan equilibrium in this respect are obviously fulfilled. The amyl alcohol is as effective a semipermeable membrane as can or-

[1] Murray, C. D., *J. Biol. Chem.*, 1923, lvi, 569.

dinarily be used in an actual experiment. In this case it is relatively impermeable to Na ions, which here play the rôle of the protein ions often used in similar studies with collodion membranes. It will be noted that in this case the relative indiffusibility of the Na ions is a condition imposed by the membrane, whereas in experiments with protein ions these ions are inherently almost non-diffusing, and therefore in many cases the membrane hardly alters the nature of the system.



Arrow shows direction of diffusion current when sign of P.D. is positive.

FIG. 1.

EXPERIMENTAL.

With the system described above several series of experiments were performed to determine the potential differences between various pairs of solutions, made up so that the condition of equilibrium de-

scribed would be satisfied. The P.D. was measured by a potentiometer in the usual manner with the use of two saturated calomel electrodes.

The *sign* is taken as positive if the direction of the positive current is from left to right through the *solutions* (as drawn or described).

All the aqueous solutions had a concentration of 0.05 N HL, with varying amounts of NaL in addition. The pH of each solution was measured electrometrically.

Solution A, the most acid of the series and containing the least amount of NaL, was placed in the beaker on the left, and Solution B on the right, and the tips of the calomel electrodes were inserted. Through the top of the Λ-tube, which is shown in the drawing, amyl alcohol containing 0.025 N HL (equilibrium concentration previously determined) was introduced. Then at the top of the Λ-tube suction was applied until the levels of the two aqueous solutions nearly reached the ridge, leaving, therefore, a small portion of amyl alcohol as a junction or membrane.

At this point the P.D. was measured. This will be designated the *alcohol junction* P.D. Finally further suction was applied to allow the two aqueous solutions to come in direct contact by just surmounting the ridge and regulating the levels so that the less dense solution surmounted the ridge first. The *aqueous junction* P.D. was then quickly measured. By lowering and raising the levels again the measurements could be repeated several times with variations within 3 mv.

Introducing fresh portions of Solution A, fresh alcohol, and substituting successively Solutions C to I in place of B, a series of P.D. was obtained for the alcohol and aqueous junctions (Series 1). Solution C was then substituted for Solution A and measured against Solutions D to I (Series 2). Finally, Solution D was measured against Solutions E to I (Series 3).

The results are given in the table. Under Series 2 there are two columns, the first gives the observed P.D. figures in millivolts as measured, and the second gives the sum of the P.D. referred to Solution A. (Thus, A *vs.* C + C *vs.* I = 63.3). Series 3 is similarly treated. It will be seen that the P.D. is nearly additive.

In addition to the series tabulated, 0.01 N NaL was measured against 0.1 N NaL, thus eliminating the HL.

0.01 NaL *vs.* 0.1 NaL (alcohol junction) = 12 mv.
0.01 " " 0.1 " (aqueous ") = −5 mv.

The results of the last mentioned experiment are indicated in Fig. 2 by the dotted slopes. It will be evident that a difference in pH of 1 in Series 1 is very nearly equivalent to a difference in pL of 1 in this last experiment. The other curves in the figure represent the P.D. obtained in Series 1, *i.e.* between Solution A of pH 2.50 and the solutions having the pH indicated by the abscissæ. The steep slope indicates the theoretical P.D. if the system followed the Donnan conditions exactly.

TABLE I.

	Solution.	pH	Series 1.	Series 2.		Series 3.	
	A	2.50	0.0				
	B	2.68	10.2				
	C	2.94	19.3	0.0	(19.3)		
	D	3.65	44.5	24.5	43.8	0.0	(44.5)
Alcohol junction.	E	3.97	47.7	31.5	50.8	3.6	48.1
	F	4.30	—	39.0	58.3	—	—
	G	4.66	54.0	44.0	63.3	13.0	57.5
	H	5.08	59.0	43.0	62.3	14.2	58.7
	I	5.50	61.0	44.0	63.3	20.0	64.5
	A	2.50	0.0				
	B	2.68	1.0				
	C	2.94	6.0	0.0	(6.0)		
	D	3.65	3.0	—	—	0.0	(3.0)
Aqueous junction.	E	3.97	0.2	—	—	−2.2	0.8
	F	4.30	—	—	—	—	—
	G	4.66	−5.2	—	—	−4.5	−1.5
	H	5.08	−7.5	—	—	−10.0	−7.0
	I	5.50	−13.0	−17.0	−11.0	−14.0	−11.0

THEORETICAL DISCUSSION.

Let us first summarize the specific properties of the system with which we are dealing. Let x and y be any two solutions such as were described in the beginning. Then, within the limits set forth in the previous article

$$HL_x = HL_y$$
$$H_x \times L_x = H_y \times L_y$$
$$pH_y - pH_x = pL_x - pL_y$$

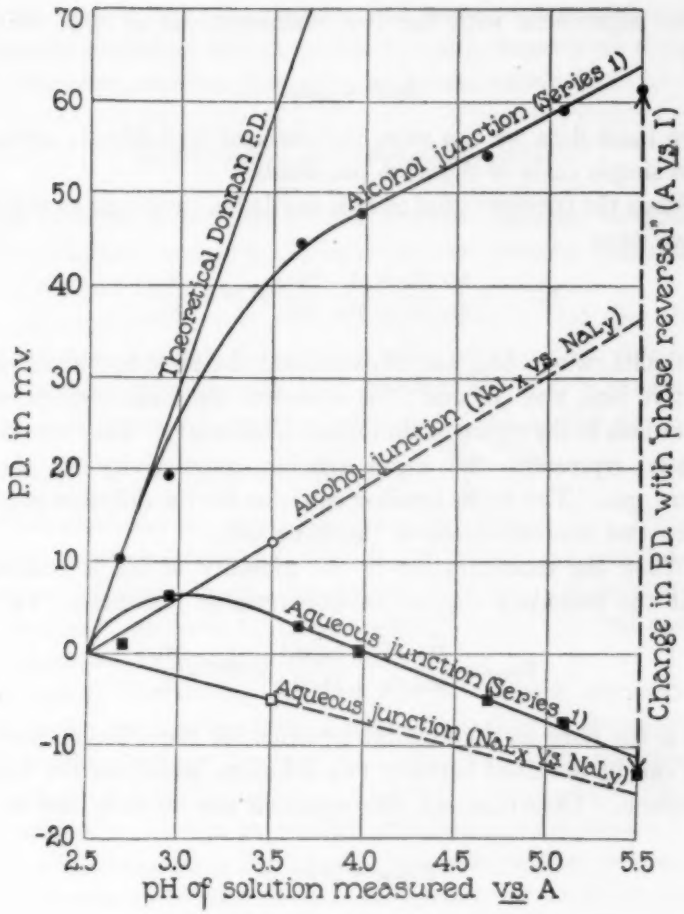


FIG. 2.

and in all but the most acid solutions, since

$$Na_x = L_x \text{ and } Na_y = L_y \text{ (very nearly)}$$

then

$$pH_y - pH_x = pNa_x - pNa_y$$

$$\frac{H_x}{H_y} = \frac{L_y}{L_x} = \frac{Na_y}{Na_x}$$

or

$$-\Delta pH_{(x-y)} = \Delta pL_{(x-y)} = \Delta pNa_{(x-y)}$$

In the experiment with the two concentrations of NaL, we have merely

$$\Delta pNa = \Delta pL$$

From these data we can write the classical approximate equations for two simple cases of diffusion potentials.[2]

A. When the concentration of H is negligible compared to the other ions, we have

$$E_{(x-y)} = \frac{M_L\,(L) - M_{Na}\,(Na)}{M_L\,(L) + M_{Na}\,(Na)} \times 59 \log \frac{Na_y}{Na_x} \qquad (1)$$

in millivolts, where M_{Na} and M_L represent the ionic mobilities of the respective ions, and (L) and (Na) represent the mean concentrations of these ions in the region of the diffusion boundary. The term M_L (L) evidently represents the mean specific conductivity of the lactate ions, etc. This is the familiar equation for the diffusion potential between two concentrations of the same salt.

B. When the concentration or the mobility of Na is reduced to zero at the boundary due to an impermeable membrane, we have

$$E_{(x-y)} = \frac{M_H\,(H) + M_L\,(L)}{M_H\,(H) + M_L\,(L)} \times 59 \log \frac{H_x}{H_y} \qquad (2)$$

which is the same as the familiar equation for the diffusion potential which can be obtained between two solutions satisfying the Donnan equilibrium. Unfortunately, this equation can be simplified to

$$E_{(x-y)} = 59 \log \frac{H_x}{H_y} \qquad (3)$$

I say "unfortunately," because many who have used equation (3) have not emphasized the derivation which Donnan used. The distinction between the conditions of equation (1) and equation (2) is that:

In the first case, the positive and negative ions tend to diffuse in the *same* direction and the net E.M.F. depends on the *difference* in their transport numbers.

[2] The derivation of the Donnan P.D. is given in Lewis, W. C. McC., A system of physical chemistry, London, 1922, ii, 320. Simple diffusion potentials are treated on p. 151.

In the second case, the positive and negative ions tend to diffuse in opposite directions and the resulting E.M.F. depends on the *sum* of their transport numbers (the sum, of course, equals 1).

Interpretation of Experimental Results.

Now in Series 1, with the alcohol junction, between the first three or four solutions equation (2) holds as the limiting condition—we have a distinct Donnan P.D.

In the experiments with two concentrations of NaL (no HL), we have a case of a simple diffusion potential and applying equation (1) it will be noted from the sign that the term $M_{Na}(Na)$ is greater than $M_L(L)$ in the *aqueous* junction, whereas in the *alcohol* junction the term $M_L(L)$ is the greater. Roughly, $\frac{M_{Na}(Na)}{M_L(L)} = 1.2$ in H_2O, $= 0.66$ in amyl alcohol, when $Na_x = L_x$, and $Na_y = L_y$. The results obtained between the last few solutions of Series 1, *i.e.* Series 3, follow the slopes indicated by the experiments with NaL solutions with the corresponding junctions and can evidently be interpreted along the lines suggested by equation (1).

But taking the alcohol junction P.D. of Series 1 over the whole range, as we measure Solution A against solutions of increasing NaL concentrations we are involved in a transition from the conditions of equation (2) (the Donnan P.D.) to those of equation (1) (the simple diffusion P.D.). Though the solubility of NaL in amyl alcohol is small, as the concentration of NaL in the aqueous solution increases, the specific conductivity due to NaL in the alcohol tends to outweigh the conductivity of the dissociated HL. As the conductivity of H ions in the boundary becomes relatively negligible we approach the conditions of equation (1).

In the case of the aqueous junctions there is at first in Series 1 a slight Donnan effect, (because the solutions are originally made up to satisfy a Donnan equilibrium as if they had been separated by an amyl alcohol membrane impermeable to Na) but obviously the increasing NaL concentrations outweigh the dissociated HL much sooner in these cases than in the cases with the alcohol junctions.

In themselves these experiments support the view advocated by Cremer[3] and more recently by Prideaux and Crooks.[4] They have convinced me that it would be more profitable to recognize the potentials occurring in such systems to be indications of a definite tendency of ions to *diffuse* from a region of high activity to one of lower activity. In all such cases, whether we have gelatin chloride in a collodion bag or NaL solution in contact with alcohol, the supposition is that (at equilibrium) the activities of the "undissociated" molecules

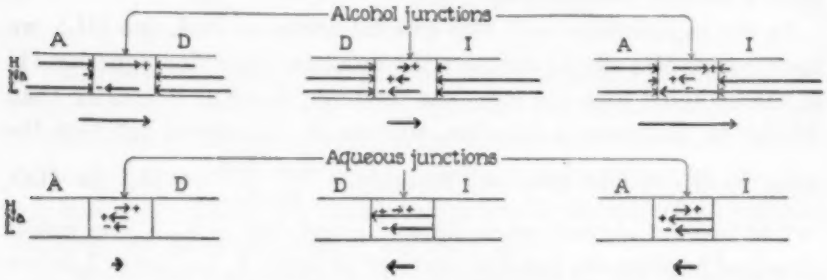


FIG. 3. These diagrams picture the diffusion process occurring in the various boundaries. The arrows in the end sections of each diagram represent roughly the concentrations in logarithmic units of H, Na, and L ions in the solutions corresponding to the letters. The bottom row, in this respect, is the same as the top row. The arrows in the central sections represent the relative tendencies to diffuse of the H, Na, and L ions. The arrow under each diagram represents the net P.D. and the direction in which the positive current tends to flow. The first diagram in the top row represents the conditions resulting from a previously established Donnan equilibrium. The central diagrams of both rows, if the H ion is neglected, represent simple diffusion conditions such as exist between two concentrations of the same salt. The other diagrams represent combinations of the two simple cases.

which can exist in both phases and migrate from one to the other must be equal in both phases so long as there is not a complete electrical circuit or a dissymmetry of membranes. This results, frequently, in the condition in which the activities of an individual ion differ in the two phases, although the activity product of the two ions which form the undissociated substance still remains equal in both phases.

[3] Cremer, M., *Z. Biol.*, 1906, xlvii, 562.

[4] Prideaux, E. B. R., and Crooks, W. E., *Chem. and Ind.*, 1923, xlii, 955.

Then, when the electrical circuit is completed, ions can move independently and the tendency is for the activities of individual ions to become equalized if the electrode P.D. is the same in the two phases (supposedly zero for saturated calomel electrodes). It is understood that, at equilibrium, what we have measured and called a diffusion potential is, under conditions where electrodes are not present, balanced by the electrostatic forces which hold oppositely charged ions together. These P.D. have usually called attention to the electrostatic condition, and the fact has been lost sight of that the source of the P.D., the source of the current (which we oppose with a battery or a charged electrometer) is due to the diffusing tendency of the ions, although this fact was well brought out by Donnan. Much has been written about "electrical double layers" and "charges" have been referred to as if they existed in aqueous solutions independently of ions. Some, too, have spoken of glass bulbs and collodion membranes acting "as hydrogen electrodes" merely because observed P.D. are proportional to the differences in pH on the two sides. This relation holds as long as the conditions of a true Donnan equilibrium are satisfied, but breaks down experimentally if the membrane becomes permeable to the ion which had been considered indiffusible, which happens usually when the concentration of this ion is large. Obviously everyone is referring to the same phenomenon, but I believe that the "kinetic" interpretation describes the processes in simpler terms and leads us more directly to an understanding of the discrepancies and anomalies in experimental results. The problem resolves itself to this general question:

A membrane, or a metal electrode, or an amalgam, or a piece of glass, all involve a second phase when in contact with a solution; what then are the thermodynamic properties of this second phase? At equilibrium what are the activities, the distribution coefficients, and the mobilities of the various components of the system in this second phase?

Incidentally, it might be mentioned that the experiments, as carried out, form an interesting example of "phase reversal." Changing from the alcohol to the aqueous junction in the last experiment listed (A *vs.* I) caused a change in P.D. from +61 mv. to −13 mv. It will be noted that in experiments with gelatin solutions removal of the collodion membrane causes no essential change in P.D. (in many cases)

because the gelatin ions are inherently non-diffusing. Many combinations of these experiments can be devised and membranes operated to simulate action currents and other bioelectric phenomena.

SUMMARY.

A system consisting of two aqueous solutions, containing equal concentrations of lactic acid, but different concentrations of Na lactate, separated by a layer of amyl alcohol has been described. This system exhibits electrical properties ranging (as the concentration of NaL is increased) from those characteristic of a simple Donnan equilibrium to those characteristic of simple diffusion.

The fact that the Donnan P.D. can be treated as a special case of a diffusion potential has been emphasized.

The experiments call attention to the effect of the thermodynamic properties of the membrane, and it is suggested that such properties as conductivities, ionic mobilities, and distribution coefficients in membranes of any sort should be investigated.

The experiments afford an interesting example of "phase reversal."

INDEX TO AUTHORS.

B

Brink, R. A. The influence of electrolytes on pollen tube development, 677

Brody, Samuel. The kinetics of senescence, 245

—. A note on the similarities between the curves of growth and of regeneration, 629

—, **Henderson, Earl W.**, and **Kempster, H. L.** The rate of senescence of the domestic fowl as measured by the decline in egg production with age, 41

— and **Ragsdale, Arthur C.** The rate of growth of the dairy cow. V. Extrauterine growth in linear dimensions, 329

—, —, and **Turner, Charles W.** The rate of growth of the dairy cow. III. The relation between growth in weight and increase of milk secretion with age, 21

—, —, and —. The rate of growth of the dairy cow. IV. Growth and senescence as measured by the rise and fall of milk secretion with age, 31

—, **Turner, Charles W.**, and **Ragsdale, Arthur C.** The relation between the initial rise and the subsequent decline of milk secretion following parturition, 541

Buchanan, R. E. See FULMER and BUCHANAN, 77

C

Caulfield, M. F. See WINSLOW, FALK, and CAULFIELD, 177

Chapman, Royal N. Nutritional studies on the confused flour beetle, *Tribolium confusum* Duval, 565

Cole, William H. The effect of laboratory age upon the phototropic reactions of *Limulus*, 295

Crozier, W. J. Hydrogen ion concentrations within the alimentary tract of insects, 289

—. On stereotropism in *Tenebrio* larvæ, 531

—. Wave length of light and photic inhibition of stereotropism in *Tenebrio* larvæ, 647

— and **Mangelsdorf, A. F.** A note on the relative photosensory effect of polarized light, 703

— and **Pilz, G. F.** The locomotion of *Limax*. I. Temperature coefficient of pedal activity, 711

D

Davis, A. R. See HOAGLAND and DAVIS, 47

E

Eggerth, Arnold H. Changes in the stability and potential of cell suspensions. I. The stability and potential of *Bacterium coli*, 63

—. II. The potential of erythrocytes, 587

Ellis, M. M. See EYSTER and ELLIS, 653

Eyster, W. H., and **Ellis, M. M.** Growth of maize seedlings as affected by glucokinin and insulin, 653

F

Falk, I. S. See WINSLOW, FALK, and CAULFIELD, 177
Freund, Jules. See NORTHROP and FREUND, 603
Fricke, Hugo. The electric conductivity of disperse systems, 741
—. A mathematical treatment of the electrical conductivity of colloids and cell suspensions, 375
Fulmer, Ellis I., and **Buchanan, R. E.** Studies on toxicity, 77

G

Gates, Frederick L. See GRANT and GATES, 635
Grant, J. H. B., and **Gates, Frederick L.** The effect on the external parathyroid glands of the exposure of rabbits to ultra-violet light, 635
Griffith, Fred R., Jr. The effect of potassium on the acid metabolism of surviving skeletal and cardiac muscles of the frog, 683

H

Hartline, H. Keffer. Influence of light of very low intensity on phototropic reactions of animals, 137
Harvey, E. Newton, and **Morrison, Thomas F.** The minimum concentration of oxygen for luminescence by luminous bacteria, 13
Hecht, Selig. Intensity discrimination and the stationary state, 355
—. Photochemistry of visual purple. III. The relation between the intensity of light and the rate of bleaching of visual purple, 731
Heidelberger, Michael. See LANDSTEINER and HEIDELBERGER, 131
Henderson, Earl W. See BRODY, HENDERSON, and KEMPSTER, 41
Heymans, C., and **Moore, A. R.** Luminescence in *Pelagia noctiluca*, 273
Hill, A. V. Membrane potentials and colloidal behavior, 91
Hill, Eben C. See MACHT and HILL, 671
Hitchcock, David I. The combination of deaminized gelatin with hydrochloric acid, 95
—. Conductivity titration of gelatin solutions with acids, 201
—. The isoelectric point of gelatin at 40°C., 457
—. Membrane potentials and colloidal behavior. Reply to the note by Professor A. V. Hill, 93
—. The solubility of tyrosine in acid and in alkali, 747
Hoagland, D. R., and **Davis, A. R.** Further experiments on the absorption of ions by plants, including observations on the effect of light, 47
Hussey, Raymond G., and **Thompson, William R.** The effect of radioactive radiations and x-rays on enzymes. II. The effect of radiations from radium emanation on pepsin in solution, 1
— and —. III. A unit of measure of activity for radium emanation, 7

K

Kempster, H. L. See BRODY, HENDERSON, and KEMPSTER, 41

Kunitz, M. A cell for the measurement of cataphoresis of ultramicroscopic particles, 413

—. Valency rule and alleged Hofmeister series in the colloidal behavior of proteins. III. The influence of salts on osmotic pressure, membrane potentials, and swelling of sodium gelatinate, 547

—. See LOEB and KUNITZ, 479

L

Landsteiner, Karl, and **Heidelberger, Michael.** Differentiation of oxyhemoglobins by means of mutual solubility tests, 131

Lasareff, P. Ionic theory of activity of nerve centers and of propagation of nerve impulse, 349

Loeb, Jacques. Hydrophilic and hydrophobic colloids and the influence of electrolytes on membrane potentials and cataphoretic potentials, 307

—. The influence of the chemical nature of solid particles on their cataphoretic P.D. in aqueous solutions, 215

—. On the location of the forces which determine the electrical double layer between collodion particles and water, 105

—. Theory of regeneration based on mass action. II, 207

—. III. Further experiments on the cause of the polar character of regeneration, 463

Loeb, Jacques, and **Kunitz, M.** The ultimate units in protein solutions and the changes which accompany the process of solution of proteins, 479

Loeb, Robert F. On the diffusibility of the calcium of blood serum through collodion membranes. The effect of sodium chloride and changes in hydrogen ion concentration, 453

Lucke, Baldwin. See MCCUTCHEON and LUCKE, 501

Lyon, Charles J. The effect of phosphates on respiration, 299

M

McCutcheon, Morton, and **Lucke, Baldwin.** The mechanism of vital staining with basic dyes, 501

Macht, David I., and **Hill, Eben C.** The effect of ultra-violet, x-ray, and radium radiation on the toxicity of normal blood, 671

Mangelsdorf, A. F. See CROZIER and MANGELSDORF, 703

Moore, A. R. The function of the brain in locomotion of the polyclad worm, *Yungia aurantiaca*, 73

—. Luminescence in *Mnemiopsis*, 403

—. The nervous mechanism of coordination in the crinoid, *Antedon rosaceus*, 281

—. See HEYMANS and MOORE, 273

Moore, Mary M. The reaction of *Cerianthus* to two sources of light, 393

—. Tropistic reactions of *Cerianthus membranaceus*, 385

Morrison, Thomas F. See HARVEY and MORRISON, 13

Murray, Cecil D. Application of the diffusion hypothesis to membrane potentials, 759

N

Northrop, John H. The kinetics of trypsin digestion. I. Experimental evidence concerning the existence of an intermediate compound, 239
—. II. Conditions under which the reaction is monomolecular, 417
—. III. The course of the reaction with constant substrate under conditions causing inactivation of the enzyme, 429
—. IV. The course of the reaction when both substrate and enzyme concentrations are decreasing, 439
—. V. Schütz's rule, 723
—. A test for diffusible ions. I. The ionic nature of trypsin, 337
— and **Freund, Jules.** The agglutination of red blood cells, 603
du Noüy, P. Lecomte. Surface tension of serum. XI. An improvement of the technique for measuring surface tension, 625

P

Parsons, Thomas R., and **Parsons, Winifred.** Observations on the transport of carbon dioxide in the blood of some marine invertebrates, 153
Parsons, Winifred. See PARSONS and PARSONS, 153
Pilz, G. F. See CROZIER and PILZ, 711
Plough, Harold H., and **Strauss, Maurice B.** Experiments on toleration of temperature by *Drosophila*, 167

R

Ragsdale, Arthur C. See BRODY and RAGSDALE, 329
—. See BRODY, RAGSDALE, and TURNER, 21, 31
—. See BRODY, TURNER, and RAGSDALE, 541
Ray, George B. The effect of cystine and glycocoll on the oxidation of sodium lactate by hydrogen peroxide, 525
—. The oxidation of sodium lactate by hydrogen peroxide, 509
Robbins, William J. Isoelectric points for the mycelium of fungi, 259

S

Shaughnessy, H. J. See WINSLOW and SHAUGHNESSY, 697
Strauss, Maurice B. See PLOUGH and STRAUSS, 167

T

Thompson, William R. See HUSSEY and THOMPSON, 1, 7
Turner, Charles W. See BRODY, RAGSDALE, and TURNER, 21, 31
—. See BRODY, TURNER, and RAGSDALE, 541

U

Uhlenhuth, Eduard. The growth of the thyroid and postbranchial body of the salamander, *Amblystoma opacum*, 597

W

Wible, Charles L. The locus of the action of veratrin, 615

Winslow, C.-E. A., Falk, I. S., and **Caulfield, M. F.** Electrophoresis of bacteria as influenced by hydrogen ion concentration and the presence of sodium and calcium salts, 177

— and **Shaughnessy, H. J.** The alkaline isopotential point of the bacterial cell, 697

INDEX TO SUBJECTS.

A

Acid(s):
Conductivity titration of gelatin solutions with (HITCHCOCK) 201
Metabolism of surviving cardiac frog muscle, effect of potassium (GRIFFITH) 683
— — — skeletal frog muscle, effect of potassium (GRIFFITH) 683
Solubility of tyrosine (HITCHCOCK) 747

Age:
Egg production, decline, as measurement of rate of senescence of domestic fowl (BRODY, HENDERSON, and KEMPSTER) 41
Milk secretion increase, relation to growth in weight (BRODY, RAGSDALE, and TURNER) 21
— —, rise and fall, as measurement of growth (BRODY, RAGSDALE, and TURNER) 31
Phototropic reactions of *Limulus*, effect on, of (COLE) 295

Agglutination:
Erythrocyte (NORTHROP and FREUND) 603

Alimentary tract:
Insects, hydrogen ion concentration (CROZIER) 289

Alkali:
Isopotential point, alkaline, of bacterial cell (WINSLOW and SHAUGHNESSY) 697

Alkali—*continued*:
Solubility of tyrosine (HITCHCOCK) 747

Ambystoma:
opacum, growth of thyroid and postbranchial body (UHLENHUTH) 597

Antedon:
rosaceus, nervous mechanism of coordination (MOORE) 281

Aurantiaca:
Yungia, function of brain in locomotion (MOORE) 73

B

Bacillus:
coli, potential of suspensions (EGGERTH) 63
—, stability of suspensions (EGGERTH) 63
—. *See also Bacterium coli.*

Bacterium:
Cell, alkaline isopotential point (WINSLOW and SHAUGHNESSY) 697
coli, potential of suspensions (EGGERTH) 63
—, stability of suspensions (EGGERTH) 63
—. *See also Bacillus coli.*
Electrophoresis, influence of hydrogen ion concentration (WINSLOW, FALK, and CAULFIELD) 177
—, — — sodium and calcium salts (WINSLOW, FALK, and CAULFIELD) 177
Luminous, minimum concentration of oxygen for

luminescence (HARVEY and MORRISON) 13

Base:
Dyes, basic, mechanism of vital staining (McCUTCHEON and LUCKE) 501

Beetle:
Confused flour, nutrition (CHAPMAN) 565

Bleaching:
Visual purple, relation of intensity of light (HECHT) 731

Blood:
Cell, red. *See* Erythrocyte.
Marine invertebrates, transport of carbon dioxide (PARSONS and PARSONS) 153
Normal, effect of radium radiation on toxicity (MACHT and HILL) 671
—, — — ultra-violet on toxicity (MACHT and HILL) 671
—, — — x-ray on toxicity (MACHT and HILL) 671
Serum. *See* Serum.

Brain:
Function in locomotion of polyclad worm (MOORE) 73
— — — — *Yungia aurantiaca* (MOORE) 73

C

Calcium:
Blood serum, diffusibility through collodion membranes (LOEB) 453
Salts, influence on electrophoresis of bacteria (WINSLOW, FALK, and CAULFIELD) 177

Carbon dioxide:
Transport, in blood of marine invertebrates (PARSONS and PARSONS) 153

Cataphoresis:
Membrane, cataphoretic, influence of electrolytes (LOEB) 307
Solid particles in aqueous solutions, influence of chemical nature on cataphoretic P. D. (LOEB) 215
Ultramicroscopic particles, cell for measurement (KUNITZ) 413

Cell:
Bacterial, alkaline isopotential point (WINSLOW and SHAUGHNESSY) 697
Blood, red. *See* Erythrocyte.
Suspensions, changes in potential (EGGERTH) 63, 587
—, — — stability (EGGERTH) 63, 587
—, electrical conductivity, mathematical treatment (FRICKE) 375

Cerianthus:
membranaceus, tropistic reactions (MOORE) 385
Reaction to two sources of light (MOORE) 393

Coli:
Bacillus, potential of suspensions (EGGERTH) 63
—, stability of suspensions (EGGERTH) 63
—. *See also Bacterium coli.*
Bacterium, potential of suspensions (EGGERTH) 63
—, stability of suspensions (EGGERTH) 63
—. *See also Bacillus coli.*

Collodion:
Membranes, diffusibility of calcium of blood serum through (LOEB) 453
Particles and water, forces which determine electrical double layer between (LOEB) 105

Colloid(s):
Behavior, and membrane potentials (HILL) 91
—, — — — (HITCHCOCK) 93
Electrical conductivity, mathematical treatment (FRICKE) 375
Hofmeister series in colloidal behavior of proteins (KUNITZ) 547
Hydrophilic (LOEB) 307
Hydrophobic (LOEB) 307
Valency rule in colloidal behavior of proteins (KUNITZ) 547

Conductivity:
Electrical, of cell suspensions, mathematical treatment (FRICKE) 375
—, — colloids, mathematical treatment (FRICKE) 375
—, disperse systems (FRICKE) 741
Titration of gelatin solutions with acids (HITCHCOCK) 201

Confusum:
Tribolium, Duval, nutrition (CHAPMAN) 565

Coordination:
Antedon rosaceus, nervous mechanism (MOORE) 281
Crinoid, nervous mechanism (MOORE) 281

Crinoid:
Coordination, nervous mechanism (MOORE) 281

Cystine:
Oxidation of sodium lactate by hydrogen peroxide, effect on, of (RAY) 525

D

Deamination:
Gelatin, deaminized, combination with hydrochloric acid (HITCHCOCK) 95

Diffusion:
Calcium of blood serum, through collodion membranes (LOEB) 453
Hypothesis, application to membrane potentials (MURRAY) 759
Ions, diffusible, test for (NORTHROP) 337

Digestion:
Trypsin, kinetics (NORTHROP) 239, 417, 429, 439, 723

Disperse system:
Conductivity, electric (FRICKE) 741

Drosophila:
Temperature toleration (PLOUGH and STRAUSS) 167

Dye:
Basic, mechanism of vital staining (McCUTCHEON and LUCKE) 501

E

Egg:
Production, senescence measured by decline with age (BRODY, HENDERSON, and KEMPSTER) 41

Electrical:
Conductivity of colloids and cell suspensions, mathematical treatment (FRICKE) 375
—, disperse systems (FRICKE) 741
Double layer between collodion particles and water, forces which determine (LOEB) 105

Electrolyte:
Cataphoretic potentials, influence on, of (LOEB) 307
Membrane potentials, influence on, of (LOEB) 307
Pollen tube development, influence on, of (BRINK) 677
Electrophoresis:
Bacteria, influence of hydrogen ion concentration (WINSLOW, FALK, and CAULFIELD) 177
—, — — sodium and calcium salts (WINSLOW, FALK, and CAULFIELD) 177
Enzyme:
Concentration, decreasing, in trypsin digestion (NORTHROP) 439
Inactivation, in trypsin digestion (NORTHROP) 429
Radioactive radiations, effect of, on (HUSSEY and THOMPSON) 1, 7
Roentgen rays, effect of, on (HUSSEY and THOMPSON) 1
Erythrocyte:
Agglutination (NORTHROP and FREUND) 603
Potential (EGGERTH) 587
Extrauterine:
Growth in dairy cow (BRODY and RAGSDALE) 329

F

Frog:
Muscle, surviving cardiac, acid metabolism of, effect of potassium (GRIFFITH) 683
—, — skeletal, acid metabolism of, effect of potassium (GRIFFITH) 683
Fungus:
Mycelium, isoelectric points (ROBBINS) 259

G

Gelatin:
Deaminized, combination with hydrochloric acid (HITCHCOCK) 95
Isoelectric point, at 40°C. (HITCHCOCK) 457
Solutions, conductivity titration with acids (HITCHCOCK) 201
Gland:
Parathyroid. *See* Parathyroid.
Glucokinin:
Growth of maize seedlings affected by (EYSTER and ELLIS) 653
Glycocoll:
Oxidation of sodium lactate by hydrogen peroxide, effect on, of (RAY) 525
Growth:
Dairy cow, rate (BRODY and RAGSDALE) 329
— —, — (BRODY, RAGSDALE, and TURNER) 21, 31
Extrauterine, in dairy cow (BRODY and RAGSDALE) 329
Maize seedlings, effect of glucokinin (EYSTER and ELLIS) 653
— —, — — insulin (EYSTER and ELLIS) 653
Measurement by rise and fall of milk secretion with age (BRODY, RAGSDALE, and TURNER) 31
Regeneration and, similarities of curves (BRODY) 629
Thyroid and postbranchial body, in *Ambystoma opacum* (UHLENHUTH) 597
— — — —, in salamander (UHLENHUTH) 597
Weight, dairy cow, relation to increase of milk secretion

with age (BRODY, RAGSDALE, and TURNER) 21

H

Heart:
Muscle, surviving cardiac, effect of potassium on acid metabolism (GRIFFITH) 683

Hofmeister series:
Colloidal behavior of proteins (KUNITZ) 547

Hydrochloric acid:
Gelatin, deaminized, combination with (HITCHCOCK) 95

Hydrogen ion:
Concentration in alimentary tract of insects (CROZIER) 289
—, effect on diffusibility of calcium of blood serum through collodion membranes (LOEB) 453
— in electrophoresis of bacteria (WINSLOW, FALK, and CAULFIELD) 177

Hydrogen peroxide:
Oxidation of sodium lactate by (RAY) 509
— — — — by, effect of cystine and glycocoll (RAY) 525

Hydrophilic:
Colloids (LOEB) 307

Hydrophobic:
Colloids (LOEB) 307

I

Inactivation:
Enzyme, in trypsin digestion (NORTHROP) 429

Inhibition:
Photic, of stereotropism in *Tenebrio* larvæ, relation of wave length of light (CROZIER) 647

Insect:
Hydrogen ion concentrations in alimentary tract (CROZIER) 289

Insulin:
Growth of maize seedlings affected by (EYSTER and ELLIS) 653

Intensity:
Discrimination, and the stationary state (HECHT) 355
Light, relation to bleaching of visual purple (HECHT) 731
—, very low intensity, influence on phototropic reactions of animals (HARTLINE) 137

Invertebrate:
Marine, carbon dioxide transport in blood (PARSONS and PARSONS) 153

Ion:
Absorption by plants (HOAGLAND and DAVIS) 47
Diffusible, test for (NORTHROP) 337
Nerve center activity, ionic theory (LASAREFF) 349
— impulse propagation, ionic theory (LASAREFF) 349
Trypsin, ionic nature (NORTHROP) 337

Isoelectric point:
Gelatin at 40°C. (HITCHCOCK) 457
Mycelium of fungi (ROBBINS) 259

Isopotential point:
Alkaline, of bacterial cell (WINSLOW and SHAUGHNESSY) 697

K

Kinetics:
Senescence (BRODY) 245
Trypsin digestion (NORTHROP) 239, 417, 429, 439, 723

L

Larva:
Tenebrio, stereotropism (CROZIER) 531
—, wave length of light and photic inhibition of stereotropism (CROZIER) 647

Light:
Absorption of ions by plants, effect on, of (HOAGLAND and DAVIS) 47
Intensity, influence on phototropic reactions of animals (HARTLINE) 137
—, relation to bleaching of visual purple (HECHT) 731
Polarized, relative photosensory effect (CROZIER and MANGELSDORF) 703
Reaction of *Cerianthus* to two sources (MOORE) 393
Ultra-violet, effect on external parathyroid glands (GRANT and GATES) 635
Wave length, and photic inhibition in stereotropism of *Tenebrio* larvæ (CROZIER) 647

Limax:
Locomotion (CROZIER and PILZ) 711

Limulus:
Age, laboratory, effect on phototropic reactions (COLE) 295

Locomotion:
Limax (CROZIER and PILZ) 711

Locomotion—*continued*:
Polyclad worm, function of brain (MOORE) 73
Yungia aurantiaca, function of brain (MOORE) 73

Luminescence:
Bacteria, luminous, minimum concentration of oxygen for (HARVEY and MORRISON) 13
Mnemiopsis (MOORE) 403
Pelagia noctiluca (HEYMANS and MOORE) 273

M

Maize:
Seedlings, effect of glucokinin on growth (EYSTER and ELLIS) 653
—, — — insulin on growth (EYSTER and ELLIS) 653

Membranaceus:
Cerianthus, tropistic reactions (MOORE) 385

Membrane:
Collodion, diffusibility of calcium of blood serum through (LOEB) 453
Potentials, application of diffusion hypothesis (MURRAY) 759
— and colloidal behavior (HILL) 91
— — — — (HITCHCOCK) 93
—, influence of electrolytes (LOEB) 307
—, — — salts on colloidal behavior of proteins (KUNITZ) 547

Metabolism:
Acid, of surviving cardiac frog muscle, effect of potassium (GRIFFITH) 683
—, — — skeletal frog muscle, effect of potassium (GRIFFITH) 683

Milk:
Secretion, growth measured by rise and fall with age (BRODY, RAGSDALE, and TURNER) 31
—, increase with age, relation to growth in weight (BRODY, RAGSDALE, and TURNER) 21
—, relation between initial rise and subsequent decline following parturition (BRODY, TURNER, and RAGSDALE) 541
—, senescence measured by rise and fall with age (BRODY, RAGSDALE, and TURNER) 31
Mnemiopsis:
Luminescence (MOORE) 403
Muscle:
Cardiac, surviving, effect of potassium on acid metabolism (GRIFFITH) 683
Skeletal, surviving, effect of potassium on acid metabolism (GRIFFITH) 683
Mycelium:
Fungi, isoelectric points (ROBBINS) 259

N

Nerve:
Centers, ionic theory of activity (LASAREFF) 349
Coordination in crinoid (MOORE) 281
Impulse, ionic theory of propagation (LASAREFF) 349
Noctiluca:
Pelagia, luminescence (HEYMANS and MOORE) 273
Nutrition:
Confused flour beetle (CHAPMAN) 565
Nutrition—*continued*:
Tribolium confusum Duval (CHAPMAN) 565

O

Opacum:
Ambystoma, growth of thyroid and postbranchial body (UHLENHUTH) 597
Osmosis:
Pressure, osmotic, influence of salts on colloidal behavior of proteins (KUNITZ) 547
Oxidation:
Sodium lactate by hydrogen peroxide (RAY) 509
— — — hydrogen peroxide, effect of cystine and glycocoll (RAY) 525
Oxygen:
Concentration, minimum for luminescence by luminous bacteria (HARVEY and MORRISON) 13
Oxyhemoglobin:
Differentiation, by mutual solubility tests (LANDSTEINER and HEIDELBERGER) 131

P

Parathyroid:
External, effect of exposure to ultra-violet light (GRANT and GATES) 635
Particle(s):
Collodion, and water, forces which determine electrical double layer between (LOEB) 105
Solid, influence of chemical nature on cataphoretic P.D. in aqueous solutions (LOEB) 215

Particle(s)—*continued*:
Ultramicroscopic, cell for measurement of cataphoresis (KUNITZ) 413
Parturition:
Milk secretion, initial rise and subsequent decline following (BRODY, TURNER, and RAGSDALE) 541
Pelagia:
noctiluca, luminescence (HEYMANS and MOORE) 273
Pepsin:
Radiations from radium emanation, effect on pepsin in solution (HUSSEY and THOMPSON) 1
Phosphate(s):
Respiration, effect on, of (LYON) 299
Photochemistry:
Visual purple (HECHT) 731
Photosensory:
Polarized light, relative effect (CROZIER and MANGELSDORF) 703
Phototropism:
Inhibition of stereotropism in *Tenebrio* larvæ, relation of wave length of light (CROZIER) 647
Limulus effect of laboratory age (COLE) 295
Reactions of animals, influence of light of very low intensity (HARTLINE) 137
Plant:
Absorption of ions (HOAGLAND and DAVIS) 47
Polarity:
Light, polarized, relative photosensory effect (CROZIER and MANGELSDORF) 703
Regeneration (LOEB) 463
Pollen:
Tube development, influence of electrolytes (BRINK) 677
Polyclad:
Locomotion, function of brain (MOORE) 73
Postbranchial body:
Growth of thyroid and, in *Ambystoma opacum* (UHLENHUTH) 597
— — — —, in salamander (UHLENHUTH) 597
Potassium:
Acid metabolism of surviving cardiac frog muscle, effect on, of (GRIFFITH) 683
— — — — skeletal frog muscle, effect on, of (GRIFFITH) 683
Potential:
Bacterium coli (EGGERTH) 63
Cataphoretic, influence of electrolytes (LOEB) 307
Cell suspensions, changes (EGGERTH) 63, 587
Difference, cataphoretic, of solid particles in aqueous solutions (LOEB) 215
Erythrocytes (EGGERTH) 587
Membrane, application of diffusion hypothesis (MURRAY) 759
—, and colloidal behavior (HILL) 91
—, — — — (HITCHCOCK) 93
—, influence of electrolytes (LOEB) 307
—, — — salts on colloidal behavior of proteins (KUNITZ) 547
Pressure:
Osmotic, influence of salts on colloidal behavior of proteins (KUNITZ) 547

Protein:
Colloidal behavior, Hofmeister series (KUNITZ) 547
— —, valency rule (KUNITZ) 547
Solution, changes accompanying (LOEB and KUNITZ) 479
—, ultimate units (LOEB and KUNITZ) 479
Purple:
Visual, photochemistry (HECHT) 731
—, relation of intensity of light to bleaching (HECHT) 731

R

Radiation:
Radioactive, effect on enzymes (HUSSEY and THOMPSON) 1, 7
Radium, effect on toxicity of normal blood (MACHT and HILL) 671
— emanation, effect on pepsin in solution (HUSSEY and THOMPSON) 1
Radioactivity:
Radiations, radioactive, effect on enzymes (HUSSEY and THOMPSON) 1, 7
Radium:
Emanation, effect of radiations on pepsin in solution (HUSSEY and THOMPSON) 1
—, unit of measure of activity (HUSSEY and THOMPSON) 7
Radiation, effect on toxicity of normal blood (MACHT and HILL) 671
Reaction:
Cerianthus, to two sources of light (MOORE) 393
Reaction—*continued*:
Phototropic, of animals, influence of light of low intensity (HARTLINE) 137
—, effect of laboratory age on *Limulus* (COLE) 295
Tropistic, *Cerianthus membranaceus* (MOORE) 385
Regeneration:
(LOEB) 207, 463
Growth and, similarities of curves (BRODY) 629
Polar character, cause (LOEB) 463
Respiration:
Phosphates, effect of, on (LYON) 299
Roentgen rays:
Enzymes, effect on, of (HUSSEY and THOMPSON) 1, 7
Toxicity, normal blood, effect on, of (MACHT and HILL) 671
Rosaceus:
Antedon, nervous mechanism of coordination (MOORE) 281

S

Salamander:
Growth of thyroid and postbranchial body (UHLENHUTH) 597
Salt(s):
Calcium, in electrophoresis of bacteria (WINSLOW, FALK, and CAULFIELD) 177
Colloidal behavior of proteins, influence on membrane potentials (KUNITZ) 547
— — — —, influence on osmotic pressure (KUNITZ) 547
— — — —, influence on swelling of sodium gelatinate (KUNITZ) 547

Salt(s)—*continued*:
Sodium, in electrophoresis of bacteria (WINSLOW, FALK, and CAULFIELD) 177
Secretion:
Milk, increase with age, relation to growth in weight (BRODY, RAGSDALE, and TURNER) 21
—, relation between initial rise and subsequent decline following parturition (BRODY, TURNER, and RAGSDALE) 541
—, rise and fall with age as measurement of growth (BRODY, RAGSDALE, and TURNER) 31
Senescence:
Domestic fowl, rate measured by decline in egg production with age (BRODY, HENDERSON, and KEMPSTER) 41
Kinetics (BRODY) 245
Measurement by rise and fall of milk secretion with age (BRODY, RAGSDALE, and TURNER) 31
Sensitivity:
Intensity discrimination and the stationary state (HECHT) 355
Serum:
Blood, calcium of, diffusibility through collodion membranes (LOEB) 453
Surface tension (DU NOÜY) 625
— —, improved technique for measuring (DU NOÜY) 625
Skeleton:
Muscle, surviving skeletal, frog, effect of potassium on acid metabolism (GRIFFITH) 683
Sodium:
Salts, influence on electrophoresis of bacteria (WINSLOW, FALK, and CAULFIELD) 177
Sodium chloride:
Concentration, effect on diffusibility of calcium of blood serum through collodion membranes (LOEB) 453
Sodium gelatinate:
Swelling, influence of salts on colloidal behavior of proteins (KUNITZ) 547
Sodium lactate:
Oxidation by hydrogen peroxide (RAY) 509
— — — —, effect of cystine and glycocoll (RAY) 525
Solubility:
Differentiation of oxyhemoglobins by mutual solubility tests (LANDSTEINER and HEIDELBERGER) 131
Tyrosine, in acid (HITCHCOCK) 747
—, — alkali (HITCHCOCK) 747
Solution:
Aqueous, solid particles in, influence of chemical nature of, on cataphoretic P. D. (LOEB) 215
Gelatin, conductivity titration with acids (HITCHCOCK) 201
Pepsin, radium emanation radiations, effect of, on (HUSSEY and THOMPSON) 1
Protein, changes accompanying (LOEB and KUNITZ) 479
—, ultimate units (LOEB and KUNITZ) 479

Stability:
Bacterium coli (EGGERTH) 63
Cell suspensions, changes (EGGERTH) 63, 587
Staining:
Vital, mechanism, with basic dyes (MCCUTCHEON and LUCKE) 501
Stereotropism:
Tenebrio larvæ (CROZIER) 531
— —, wave length of light and photic inhibition (CROZIER) 647
Suspension:
Cell, changes in potential (EGGERTH) 63, 587
—, — — stability (EGGERTH) 63, 587
—, electrical conductivity, mathematical treatment (FRICKE) 375

T

Tenebrio:
Larvæ, stereotropism (CROZIER) 531
—, —, wave length of light and photic inhibition (CROZIER) 647
Tension:
Surface, of serum (DU NOÜY) 625
—, — —, improved technique for measuring (DU NOÜY) 625
Test:
Mutual solubility, differentiation of oxyhemoglobins (LANDSTEINER and HEIDELBERGER) 131
Thyroid:
Growth of postbranchial body and, in *Ambystoma opacum* (UHLENHUTH) 597
— — — — and, in salamander (UHLENHUTH) 597
Titration:
Conductivity, gelatin solutions with acids (HITCHCOCK) 201
Toxicity:
(FULMER and BUCHANAN) 77
Normal blood, effect of radium radiation (MACHT and HILL) 671
— —, — — ultra-violet (MACHT and HILL) 671
— —, — — x-ray (MACHT and HILL) 671
Tribolium:
confusum Duval, nutrition (CHAPMAN) 565
Tropism:
Cerianthus membranaceus (MOORE) 385
Trypsin:
Digestion, kinetics (NORTHROP) 239, 417, 429, 439, 723
Ionic nature (NORTHROP) 337
Tyrosine:
Solubility in acid (HITCHCOCK) 747
— — alkali (HITCHCOCK) 747

U

Ultramicroscopic:
Particles, cell for measurement of cataphoresis (KUNITZ) 413
Ultra-violet:
Light, effect on external parathyroid glands (GRANT and GATES) 635
Rays, effect on toxicity of normal blood (MACHT and HILL) 671

V

Valency:
Rule in colloidal behavior of proteins (KUNITZ) 547
Veratrin:
Locus of action (WIBLE) 615
Vital staining:
Mechanism, with basic dyes (MCCUTCHEON and LUCKE) 501

W

Water:
Electrical double layer between collodion particles and water, location of forces which determine (LOEB) 105
Water—*continued*:
Particles, solid, influence of chemical nature on cataphoretic P. D. in aqueous solutions (LOEB) 215
Weight:
Growth in dairy cow, relation to increase of milk secretion with age (BRODY, RAGSDALE, and TURNER) 21
Worm:
Polyclad, function of brain in locomotion (MOORE) 73

Y

Yungia:
aurantiaca, function of brain in locomotion (MOORE) 73

MONOGRAPHS OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

Under the head of *Monographs of The Rockefeller Institute for Medical Research* are published from time to time scientific papers which are so extensive, or require such elaborate illustrations, as to render them unsuitable for current periodical issues. The *Monographs* are published at irregular periods, determined by the available material on hand. A number of free copies, which may be 50 or less according to the size of the *Monograph*, are given to the author. *Monographs* will be sent post paid on application, payable in advance. Remittances should be made by draft or check on New York, or by postal money order, payable to The Rockefeller Institute for Medical Research, Avenue A and 66th Street, New York, N. Y.

No. 1. Simon Flexner, J. W. Jobling, and Maud L. Menten. Experimental studies on tumor. Plates 1–28. (Issued June 30, 1910.) Price, $1.00.

No. 2. William Travis Howard and Oscar T. Schultz. Studies in the biology of tumor cells. Plates 1–6. (Issued February 15, 1911.) Price, $1.00.

No. 3. Benjamin T. Terry. Chemo-therapeutic trypanosome studies with special reference to the immunity following cure. (Issued March 15, 1911.) Price, $1.00.

No. 4. Francis W. Peabody, George Draper, and A. R. Dochez. A clinical study of acute poliomyelitis. Plates 1–13. (Issued June 24, 1912.) Price, $1.00.

No. 5. J. P. Simonds. Studies in *Bacillus welchii*, with special reference to classification and to its relation to diarrhea. (Issued September 27, 1915.) Price, $1.00.

No. 6. James L. Stoddard and Elliott C. Cutler. Torula infection in man. Plates 1–9. (Issued January 31, 1916.) Price, $1.00.

No. 7. Oswald T. Avery, H. T. Chickering, Rufus Cole, and A. R. Dochez. Acute lobar pneumonia. Prevention and serum treatment. Plates 1–3. (Issued October 16, 1917.) Price, $1.00.

No. 8. Ernest C. Dickson. Botulism. A clinical and experimental study. Plates 1–10. (Issued July 31, 1918.) Price, $1.00.

No. 9. James Howard Brown. The use of blood agar for the study of streptococci. Plates 1–34. (Issued January 21, 1919.) Price, $2.00.

No. 10. William G. MacCallum. The pathology of the pneumonia in the United States Army camps during the winter of 1917–18. Plates 1–53. (Issued April 16, 1919.) Price, $1.50.

No. 11. Frederick M. Allen, Edgar Stillman, and Reginald Fitz. Total dietary regulation in the treatment of diabetes. 62 charts. (Issued October 15, 1919.) Price, $4.50.

No. 12. Lewis H. Weed, Paul Wegeforth, James B. Ayer, and Lloyd D. Felton. A study of experimental meningitis. A series of papers from the Army Neuro-Surgical Laboratory. Plates 1–17. (Issued March 25, 1920.) Price, $1.00.

No. 13. Werner Marchand. The early stages of Tabanidæ (horse-flies). Plates 1–15. (Issued November 15, 1920.) Price, $2.00.

No. 14. S. T. Darling and W. G. Smillie. Studies on hookworm infection in Brazil. First paper. (Issued February 1, 1921.) Published for the International Health Board.

No. 15. I. J. Kligler. Investigation on soil pollution and the relation of the various types of privies to the spread of intestinal infections. (Issued October 10, 1921.) Published for the International Health Board.

No. 16. Abraham L. Garbat. Typhoid carriers and typhoid immunity. (Issued May 10, 1922.) Price, $1.25.

No. 17. Wilson G. Smillie. Studies on hookworm infection in Brazil. Second paper. (Issued May 12, 1922.) Published for the International Health Board.

No. 18. P. A. Levene. Hexosamines, their derivatives, and mucins and mucoids. (Issued July 7, 1922.) Price, $1.25.

No. 19. Theobald Smith and Ralph B. Little. Studies in vaccinal immunity towards disease of the bovine placenta due to *Bacillus abortus* (infectious abortion). (Issued November 15, 1923.) Price, $1.25.

No. 20. Hideyo Noguchi *et al.* Experimental studies of yellow fever in northern Brazil. Plates 1–5. (To be issued about August 1, 1924.) Published for the International Health Board.

CONTENTS

DU NOÜY, P. LECOMTE. Surface tension of serum. XI. An improvement of the technique for measuring surface tension. Plate 1. 625

BRODY, SAMUEL. A note on the similarities between the curves of growth and of regeneration 629

GRANT, J. H. B., and GATES, FREDERICK L. The effect on the external parathyroid glands of the exposure of rabbits to ultra-violet light. Plate 2 635

CROZIER, W. J. Wave length of light and photic inhibition of stereotropism in *Tenebrio* larvæ 647

EYSTER, W. H., and ELLIS, M. M. Growth of maize seedlings as affected by glucokinin and insulin 653

MACHT, DAVID I., and HILL, EBEN C. The effect of ultra-violet, x-ray, and radium radiation on the toxicity of normal blood 671

BRINK, R. A. The influence of electrolytes on pollen tube development 677

GRIFFITH, FRED R., JR. The effect of potassium on the acid metabolism of surviving skeletal and cardiac muscles of the frog 683

WINSLOW, C.-E. A., and SHAUGHNESSY, H. J. The alkaline isopotential point of the bacterial cell 697

CROZIER, W. J., and MANGELSDORF, A. F. A note on the relative photosensory effect of polarized light 703

CROZIER, W. J., and PILZ, G. F. The locomotion of *Limax*. I. Temperature coefficient of pedal activity 711

NORTHROP, JOHN H. The kinetics of trypsin digestion. V. Schütz's rule 723

HECHT, SELIG. Photochemistry of visual purple. III. The relation between the intensity of light and the rate of bleaching of visual purple 731

FRICKE, HUGO. The electric conductivity of disperse systems 741

HITCHCOCK, DAVID I. The solubility of tyrosine in acid and in alkali 747

MURRAY, CECIL D. Application of the diffusion hypothesis to membrane potentials 759

INDEX TO VOLUME VI 769

The Journal of General Physiology is issued bimonthly, one volume of about 600 pages appearing in a year. Contributions should be sent to the editors of *The Journal of General Physiology*, Avenue A and 66th Street, New York, N. Y., or 60 Buckingham Street, Cambridge, Mass. The papers should be limited preferably to twenty printed pages, not counting space occupied by illustrations. Authors receive 100 reprints of their papers free of charge; additional copies may be obtained at cost.

The subscription price per year (one volume) is $5.00, payable in advance. Single copies cost $1.00. Remittances should be made by draft or check on New York, or by postal money order, payable to *The Journal of General Physiology*, Mount Royal and Guilford Avenues, Baltimore, Md., or Avenue A and 66th Street, New York, N. Y.